Supporting Information

Synthesis and Structure-Activity Relationships of *N*-Dihydrocoptisine-8-ylidene Aromatic Amines and *N*-Dihydrocoptisine-8-ylidene Aliphatic Amides as Antiulcerative Colitis Agents Targeting XBP1

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CONTENTS

Cytotoxicity assay on IEC-6 cells. IEC-6 cells at high confluence (>90%) were digested with 0.25% trypsin/0.1% EDTA and then seeded in a 96-well plate at 2×103 cells/well. The medium was discarded the next day, and the cells were cultivated with tested compounds at a final concentration of 10 μM. The cytotoxicity was measured by an MTT assay (n = 5) 0, 24, 48, and 72 h after co-culturing IEC-6 cells with test compounds.

Dual Luciferase Reporter Assay. IEC-6 cells in the growth phase were dispersed in 48-well plates at a density of 5 x 104 cells/well, and the plates were placed in a humidified incubator filled with 5% CO2 at 37 °C. Plasmid transfection (0.6 μg/well) was performed for 4 h when the cell confluence reached 70-80%. Compounds at a final concentration of 10 μM were added into the wells (n = 3) after 4 h of transfection and were co-incubated for an additional 48 h. Luciferase activity was detected using a dual luciferase reporter gene detection kit (Promega, USA).

EC_{50} Values Assay. IEC-6 cells in the growth phase were dispersed in 48-well plates at a density of 5 x 104 cells/well, and the plates were placed in a humidified incubator filled with 5% CO2 at 37 °C. Plasmid transfection (0.6 μg/well) was performed for 4 h when the cell confluence reached 70-80%. Compounds 1, 5, 6, and 7 were added into these wells (n = 3) in a concentration gradient after 4 h of transfection and then co-incubated for an additional 48 h. Luciferase activity was detected using a dual luciferase reporter gene detection kit (Promega, USA).

Animals. C57bl/6j mice with the range of body weight of 18-22 g and Sprague–Dawley (SD) rats with the range of body weight of 180-220g were obtained from Vital River Laboratories. All of the animal experiments in this study were carried out in accordance with approved protocols of the experimental animal research center. The experimental procedures were approved by the Institutional Ethical Committee for Animal Care and Use of Chinese Academy of Medical Sciences (CAMS).

Animal experiments of 2 and (±)-5a on C57bl/6j mouse model with DSS-induced acute UC. C57bl/6j mice were administrated orally with 2.3% DSS in drinking water for 7 days to generate the acute UC model. The success of generating UC model was indicated by the biomarkers of the loss of body weight, the decreases of DAI and CMDI, and the obvious contracture of the colons as compared with the normal control group. From the first day after the UC model generation on, SASP of 300 mg/kg dose, compound 2 of 200 mg/kg, 100 mg/kg, and 50 mg/kg doses and (±)-5a of 300 mg/kg, 150 mg/kg, and 75 mg/kg doses were given to the corresponding groups orally in the form of solid dispersion once a day, respectively. On the eighth day of administration, all the experiment mice were sacrificed, and the last lengths of colon of every mouse 8 cm were excised, opened longitudinally, and rinsed with normal saline solution. Then, the tissue samples were taken for macroscope, DAI, and CMDI scoring, histopathologic examination, and IR (%) calculation. The IR (%) of administration groups and positive control group on DAI was calculated by the following formula.

$$IR(\%) = \frac{DAI(M) - DAI(AorP)}{DAI(M)} \times 100\%$$

DAI (M) means the DAI value of model group, while DAI (A or P) means the DAI value of administration groups or positive control group.

(A) Figures

Figure S1. ^{1}H NMR (DMSO-d_6, 400 MHz) spectrum of **2**.

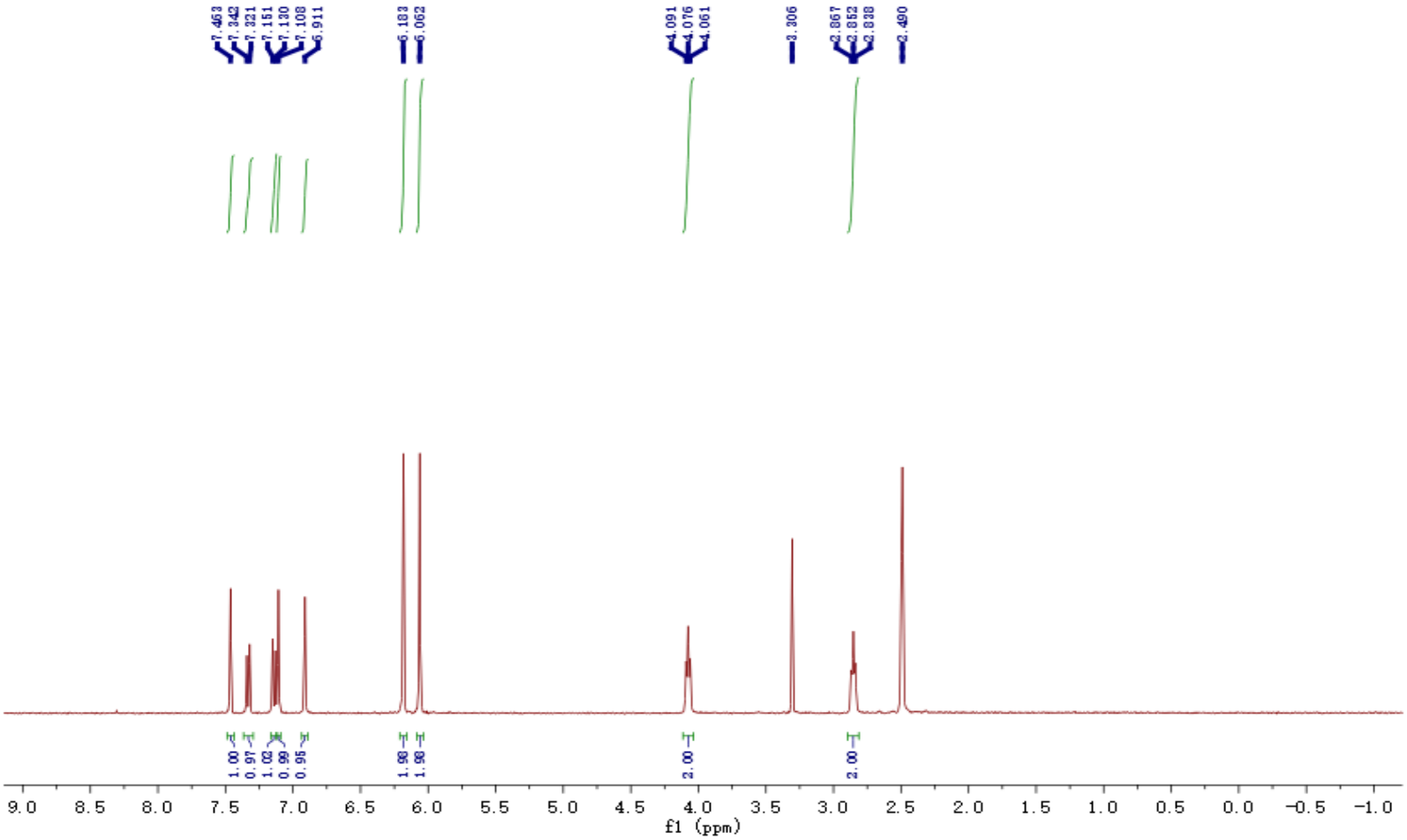

Figure S2. ^{13}C NMR (DMSO-d_6, 150 MHz) spectrum of **2**.

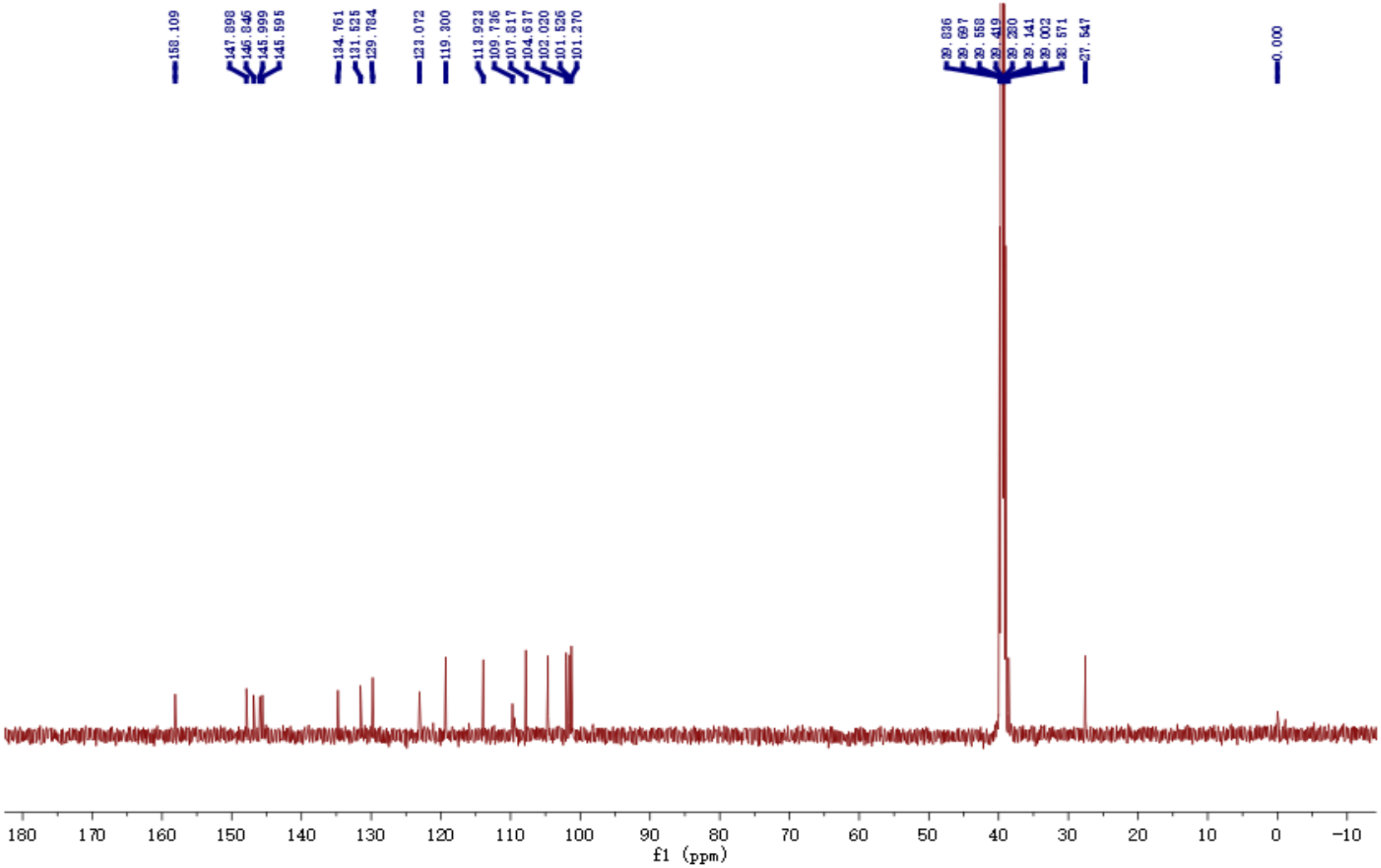

Figure S3. ^{1}H NMR (DMSO-d_6, 400 MHz) spectrum of **3**.

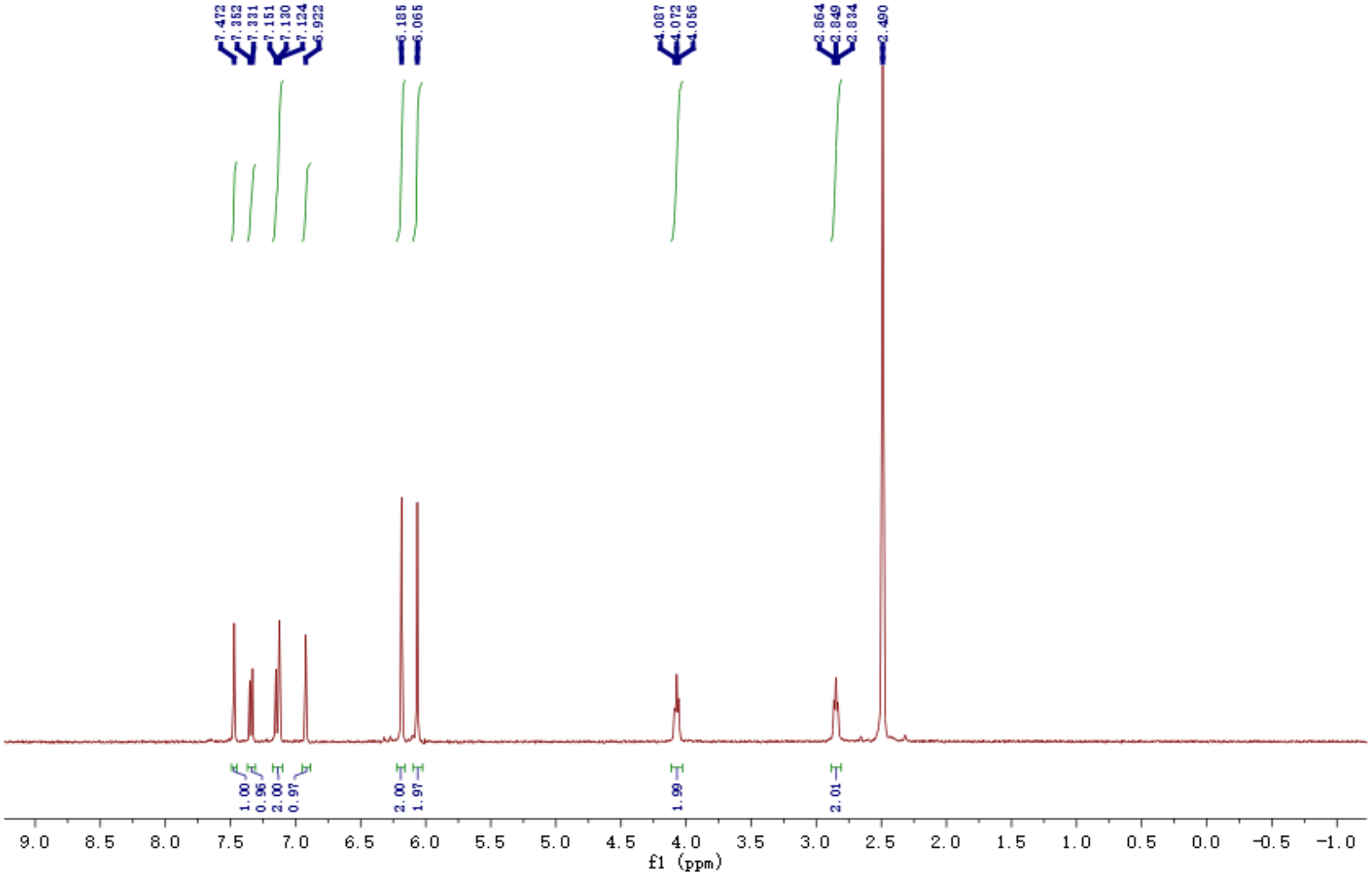

Figure S4. ^{1}H NMR (DMSO-d_6, 400 MHz) spectrum of **4a**.

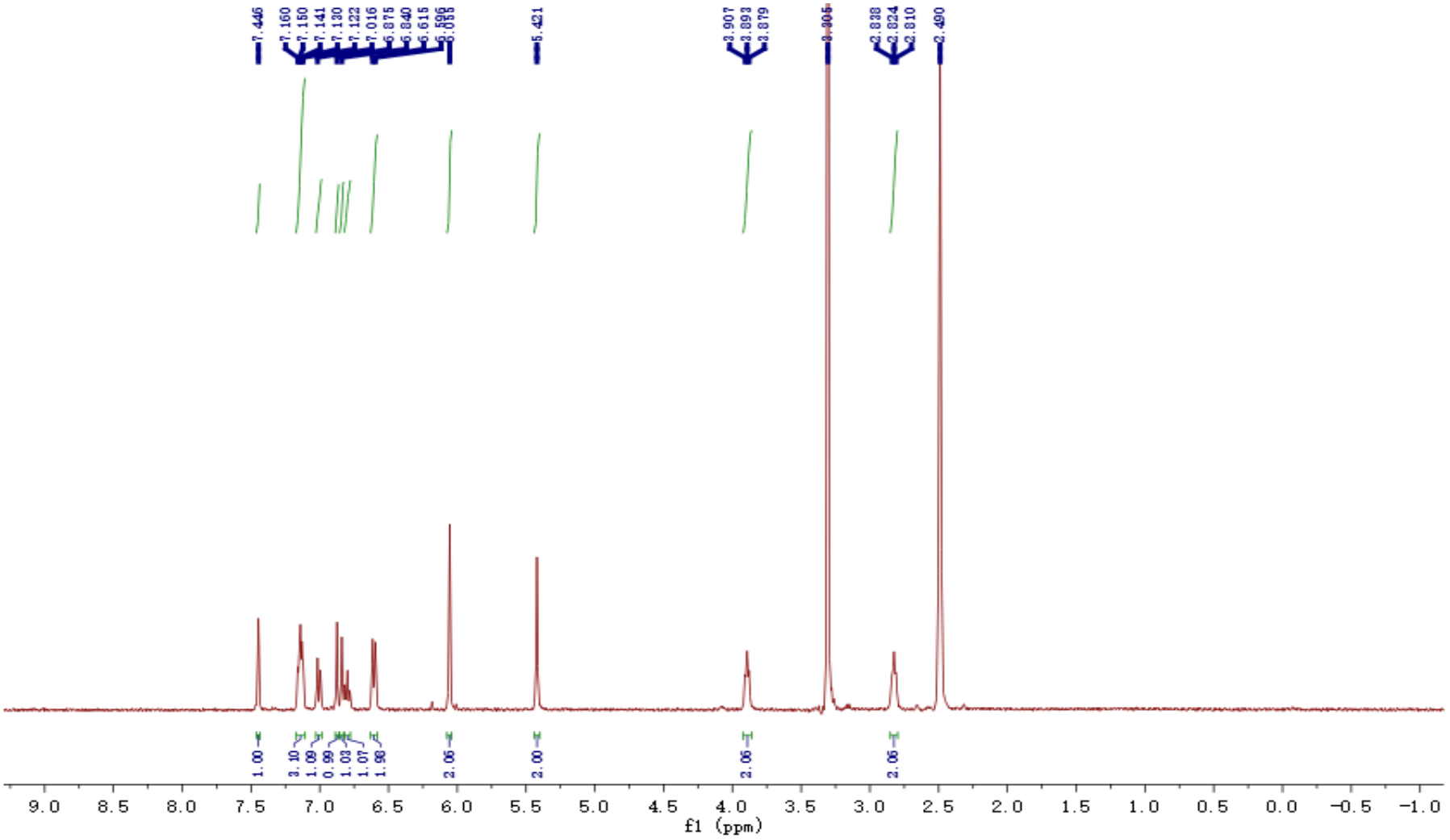

Figure S5. ^{13}C NMR (DMSO-d_6, 150 MHz) spectrum of **4a**.

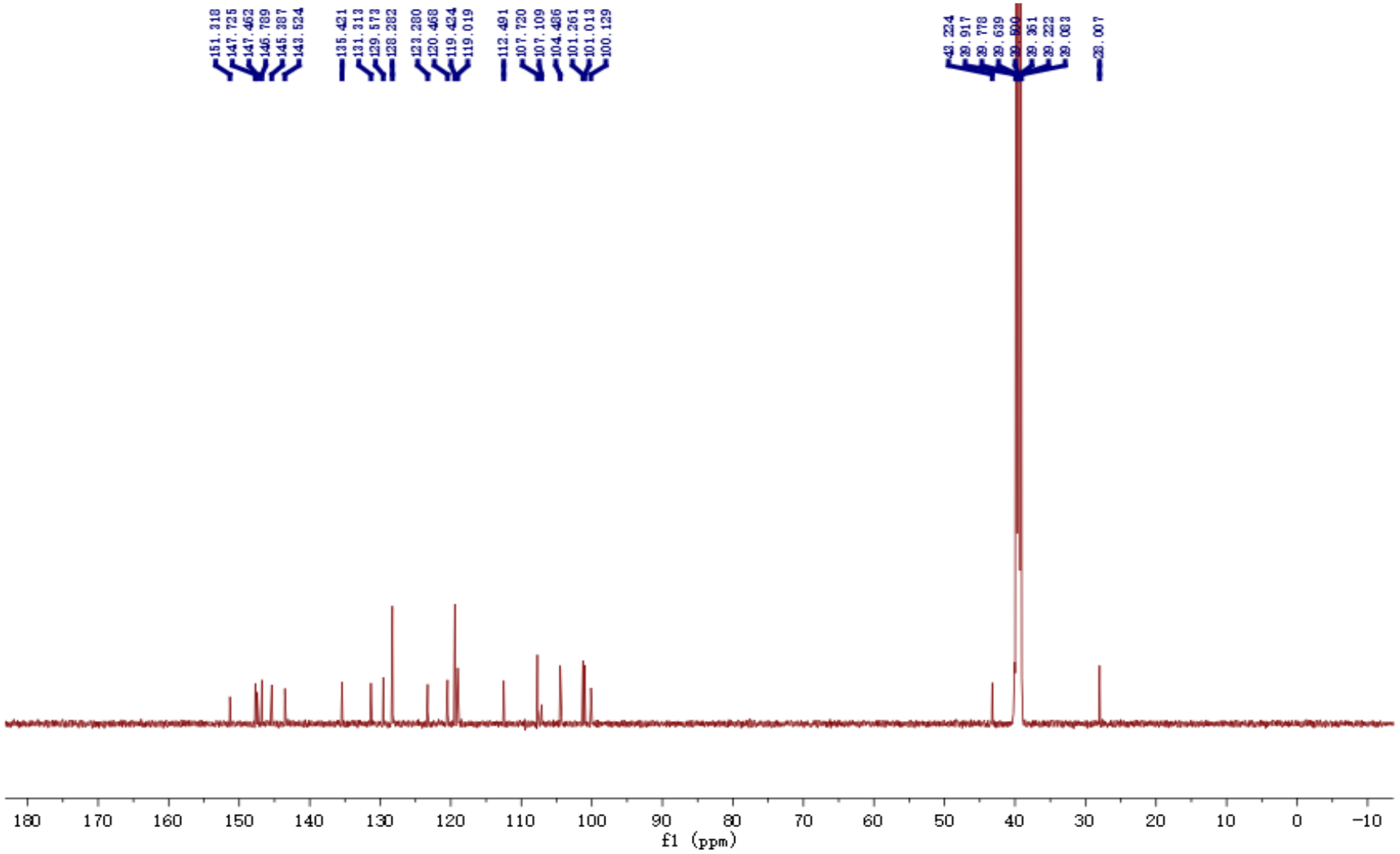

Figure S6. ^{1}H NMR (DMSO-d_6, 400 MHz) spectrum of **4b**.

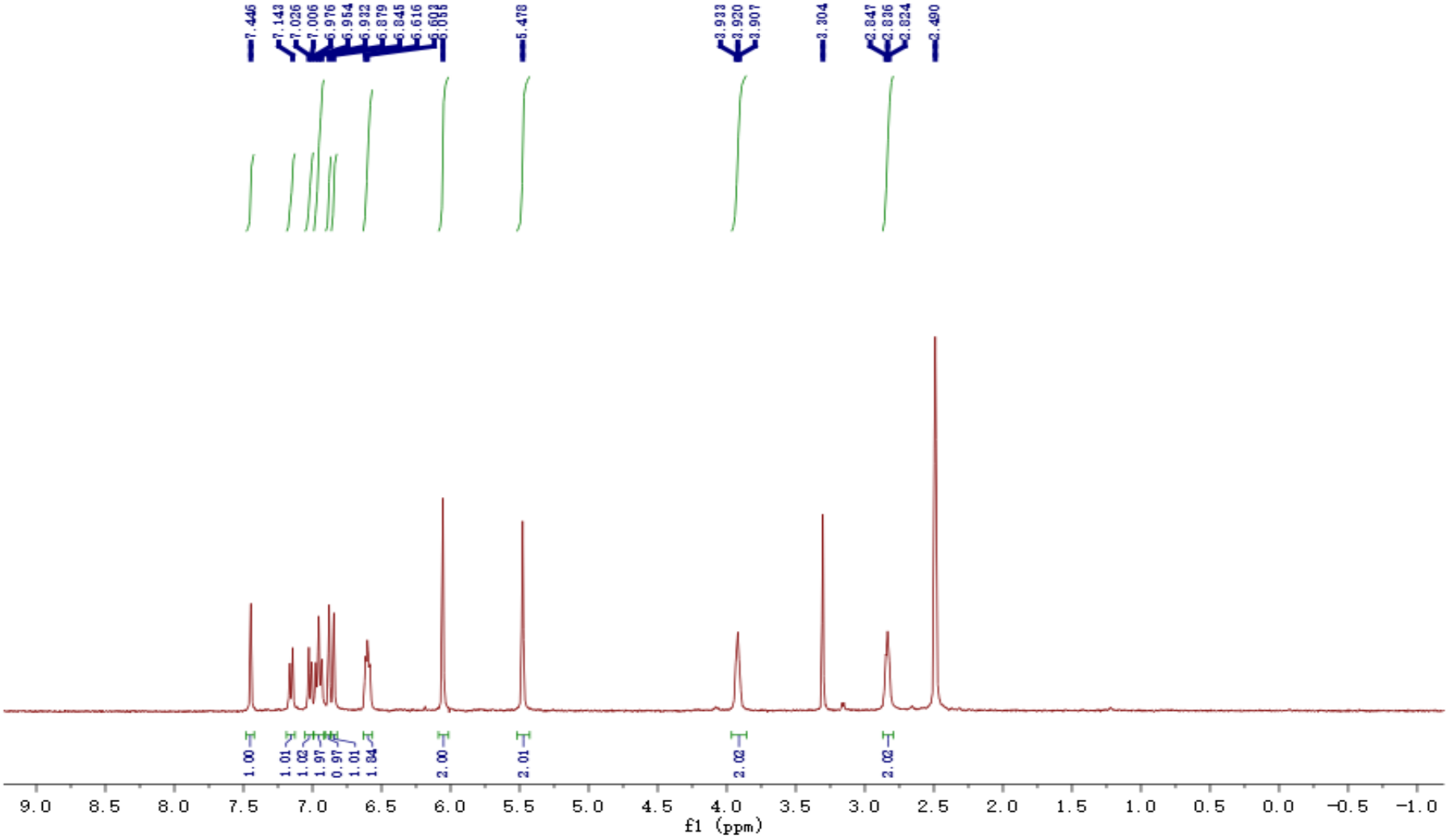

Figure S7. ^{13}C NMR (DMSO-d_6, 150 MHz) spectrum of **4b**.

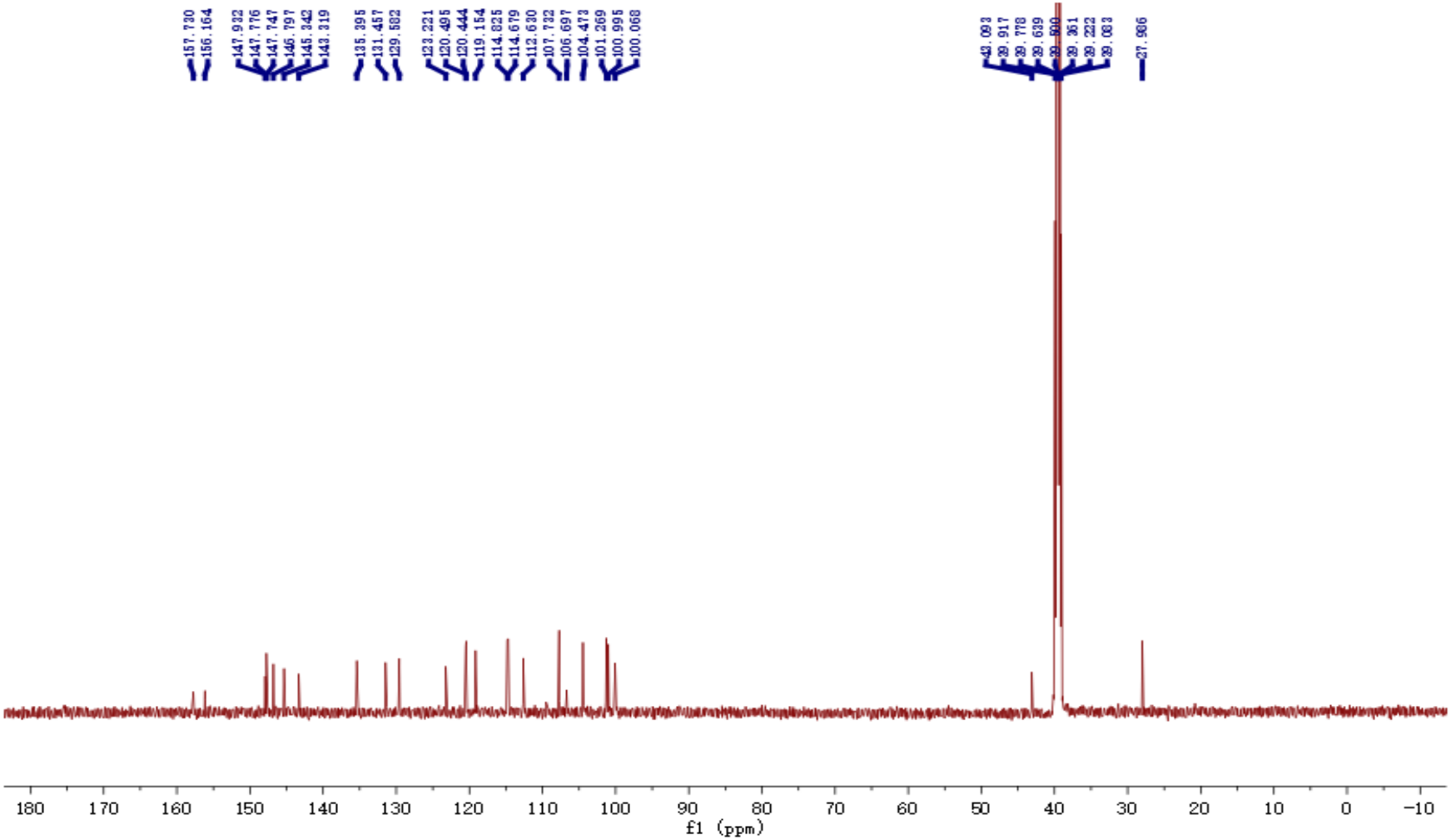

Figure S8. ^{1}H NMR (DMSO-d_6, 400 MHz) spectrum of **4c**.

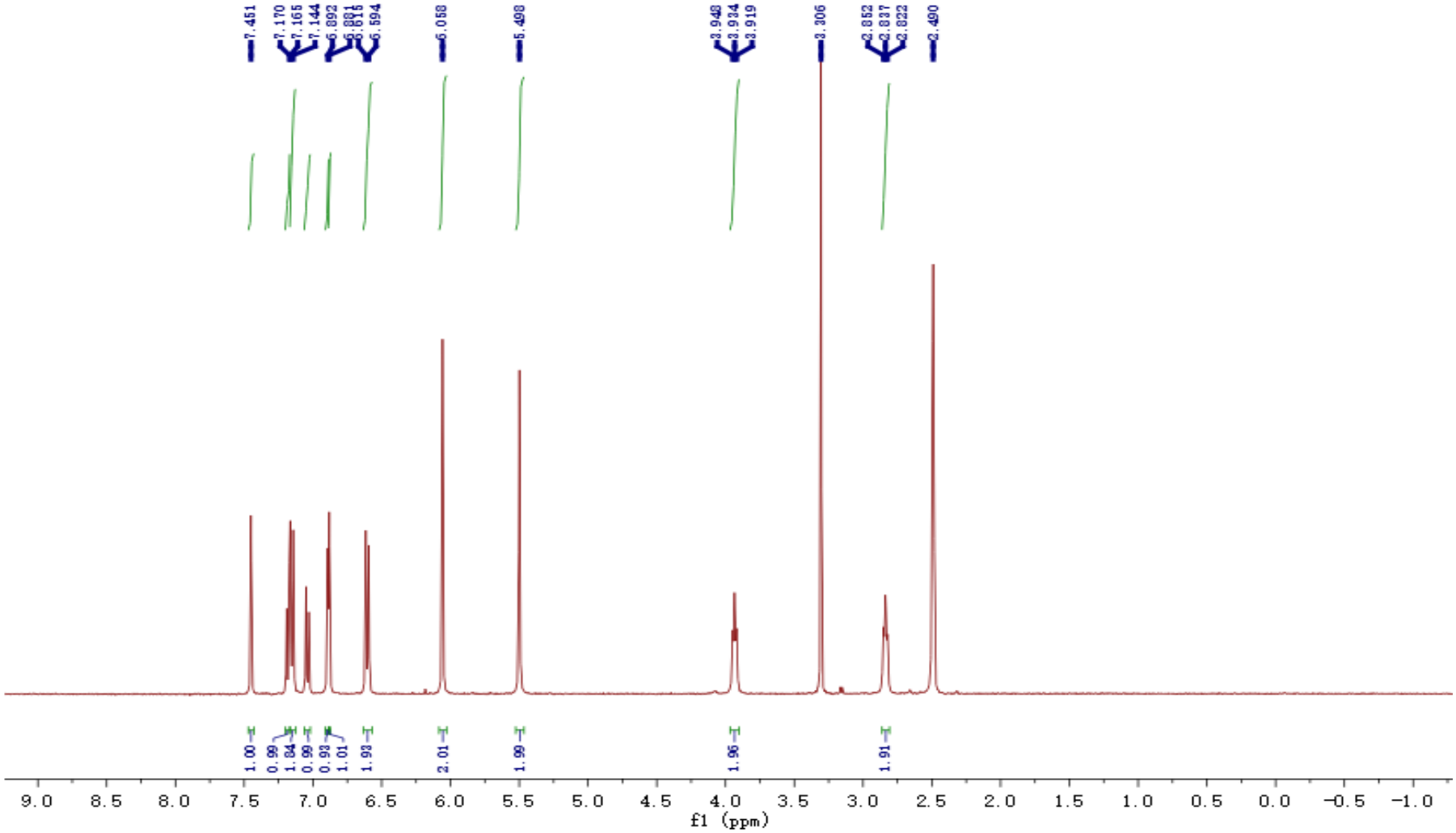

Figure S9. ^{13}C NMR (DMSO-d_6, 150 MHz) spectrum of **4c**.

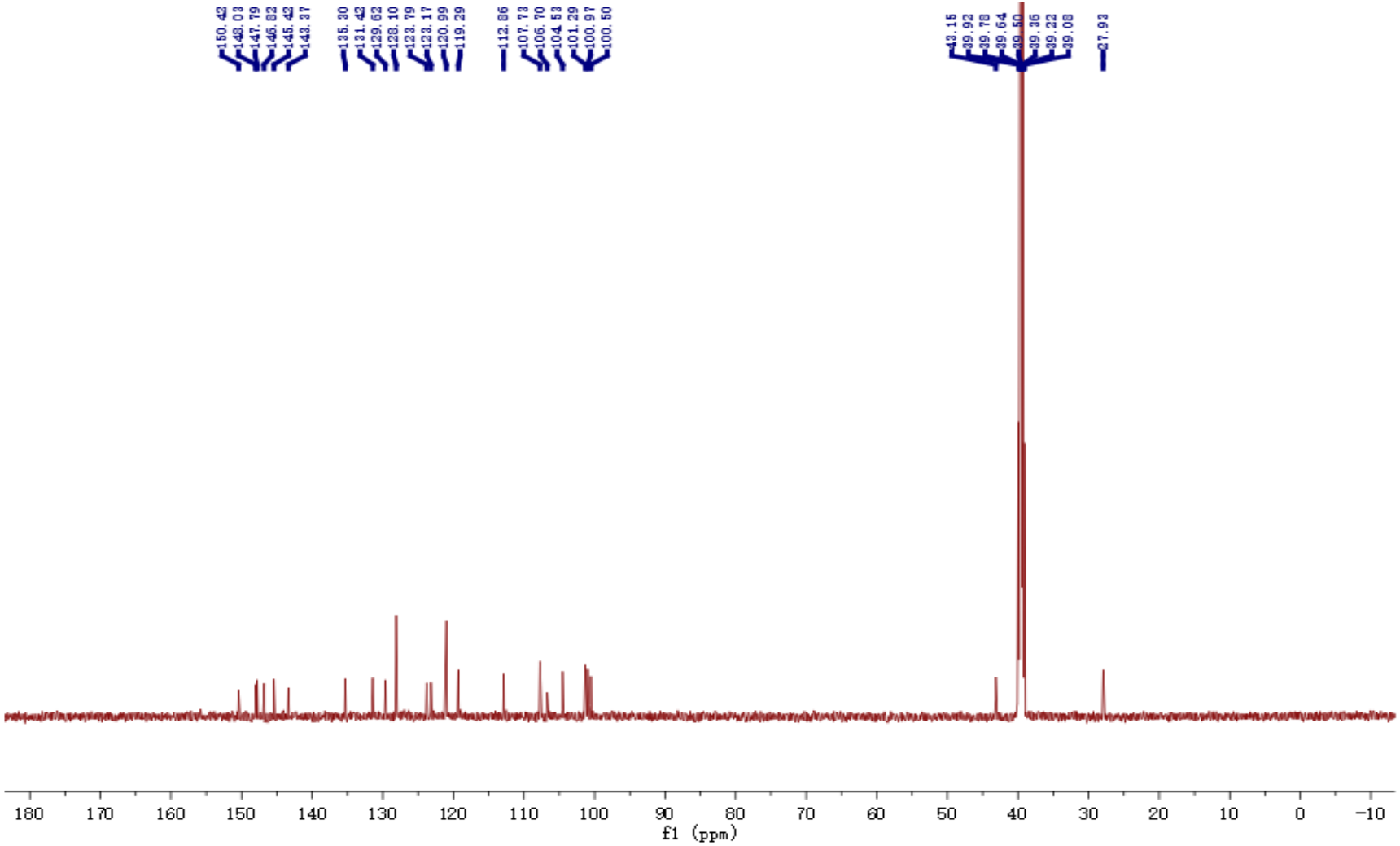

Figure S10. ^{1}H NMR (DMSO-d_6, 400 MHz) spectrum of **4d**.

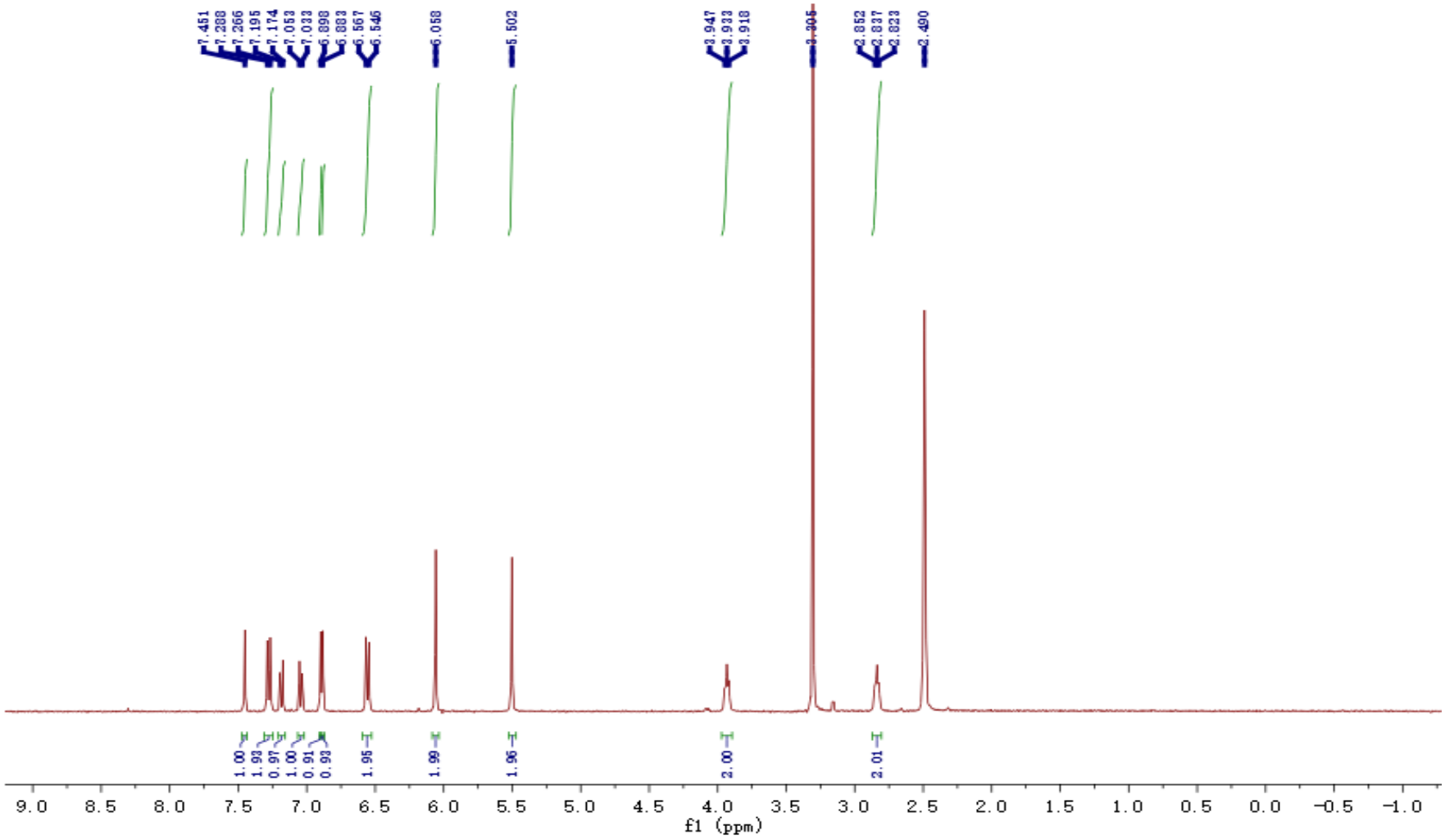

Figure S11. ^{13}C NMR (DMSO-d_6, 150 MHz) spectrum of **4d**.

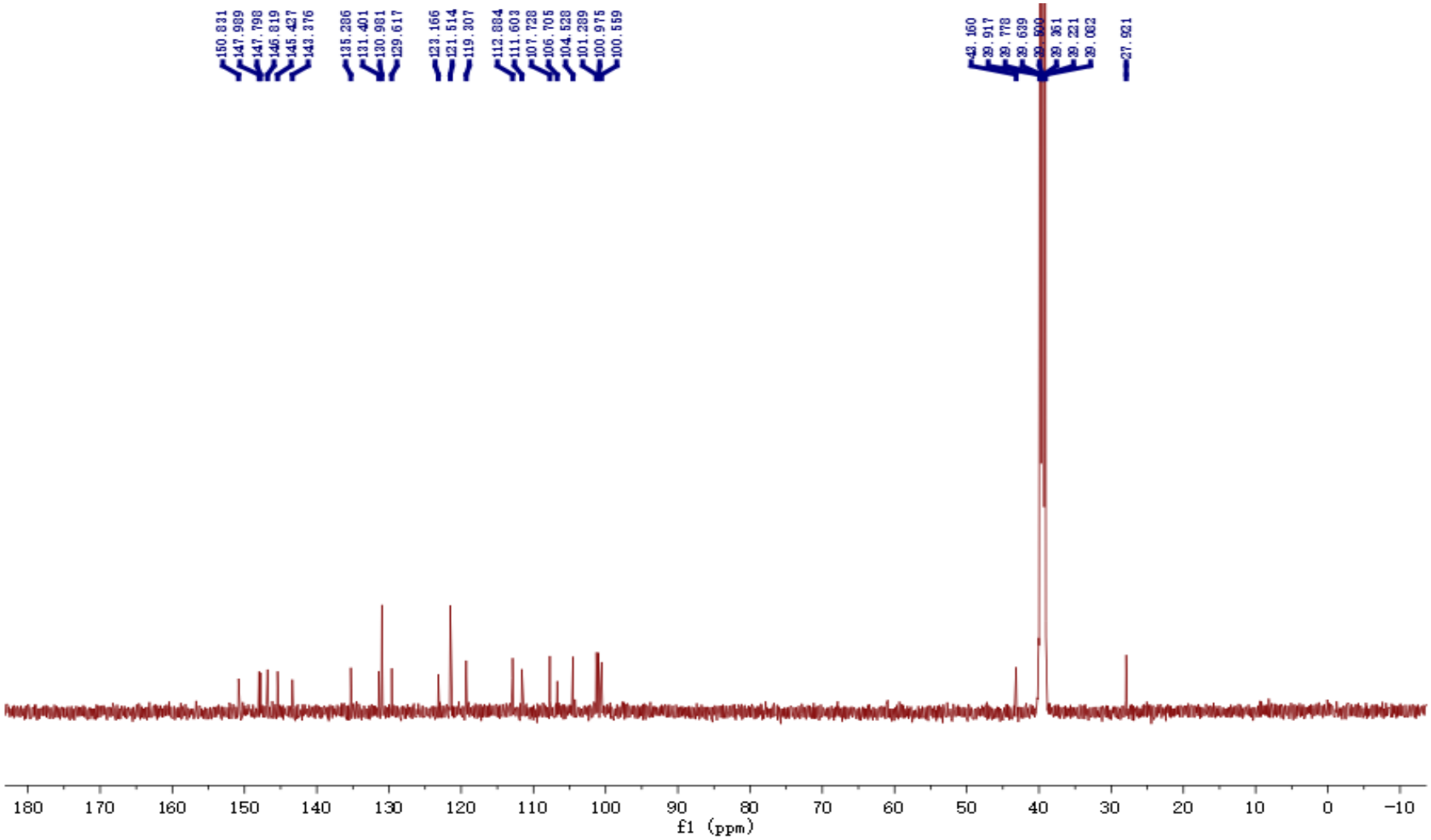

Figure S12. ^{1}H NMR (DMSO-d_6, 400 MHz) spectrum of **4e**.

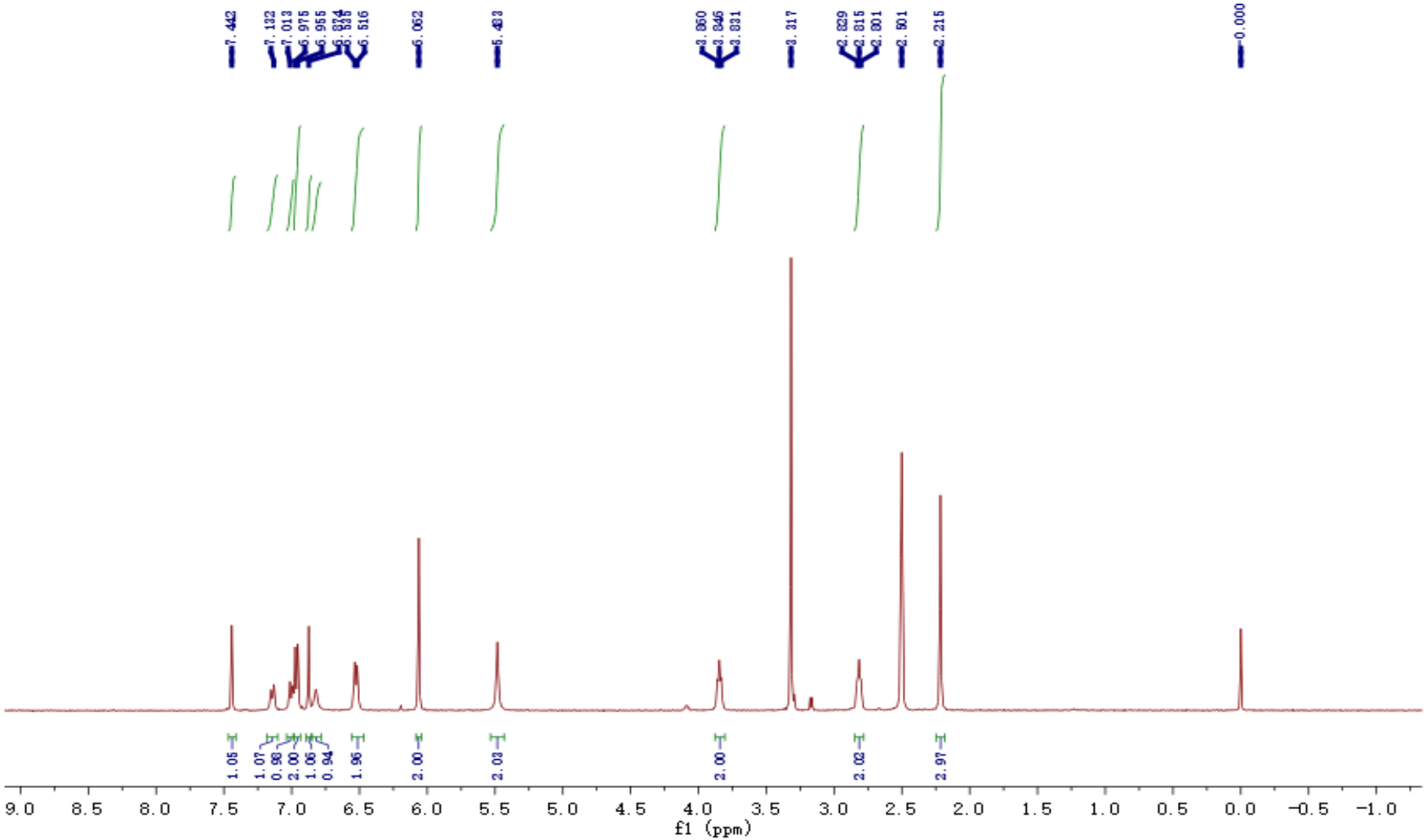

Figure S13. ^{13}C NMR (DMSO-d_6, 100 MHz) spectrum of **4e**.

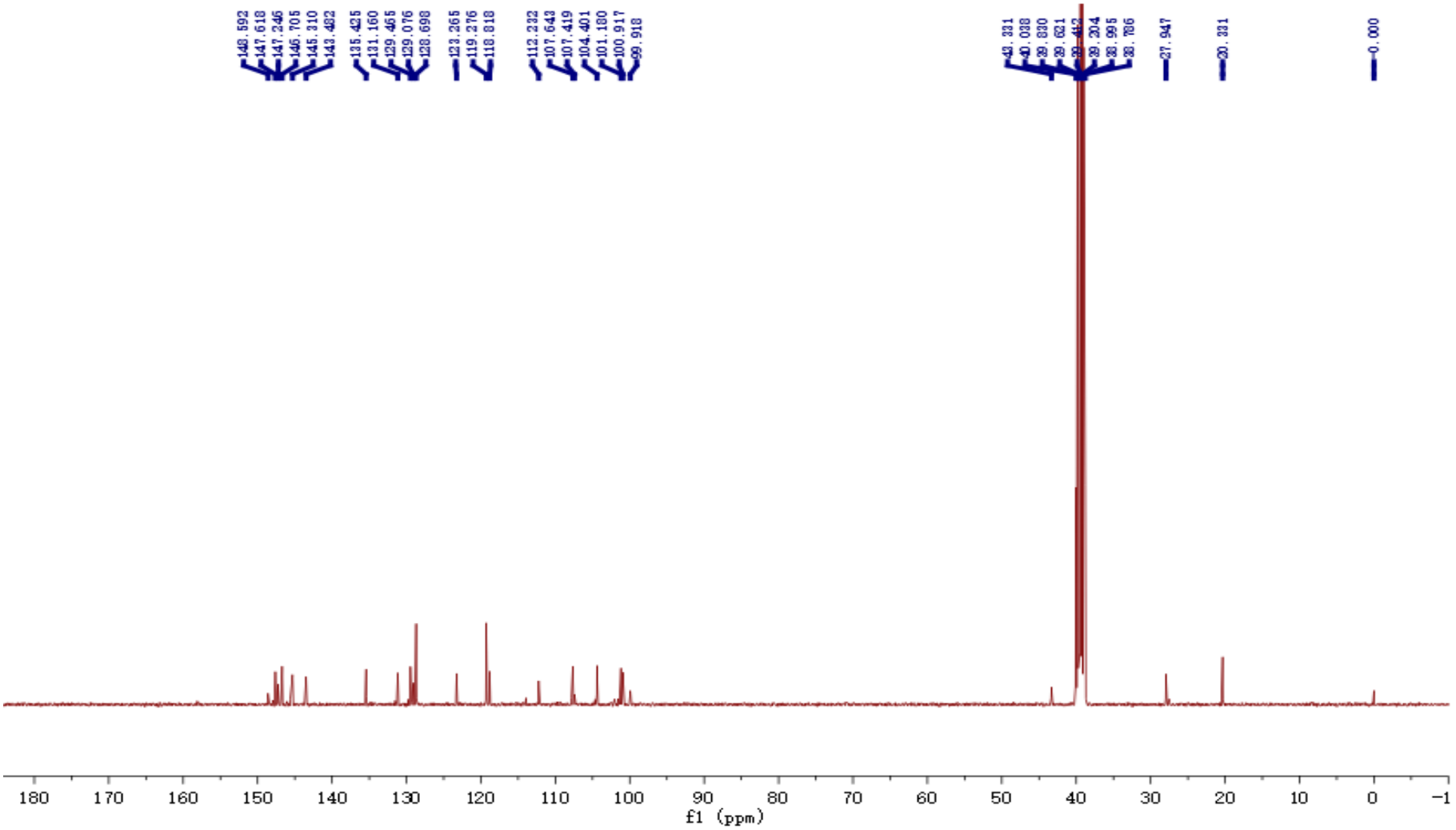

Figure S14. ^{1}H NMR (DMSO-d_6, 400 MHz) spectrum of **4f**.

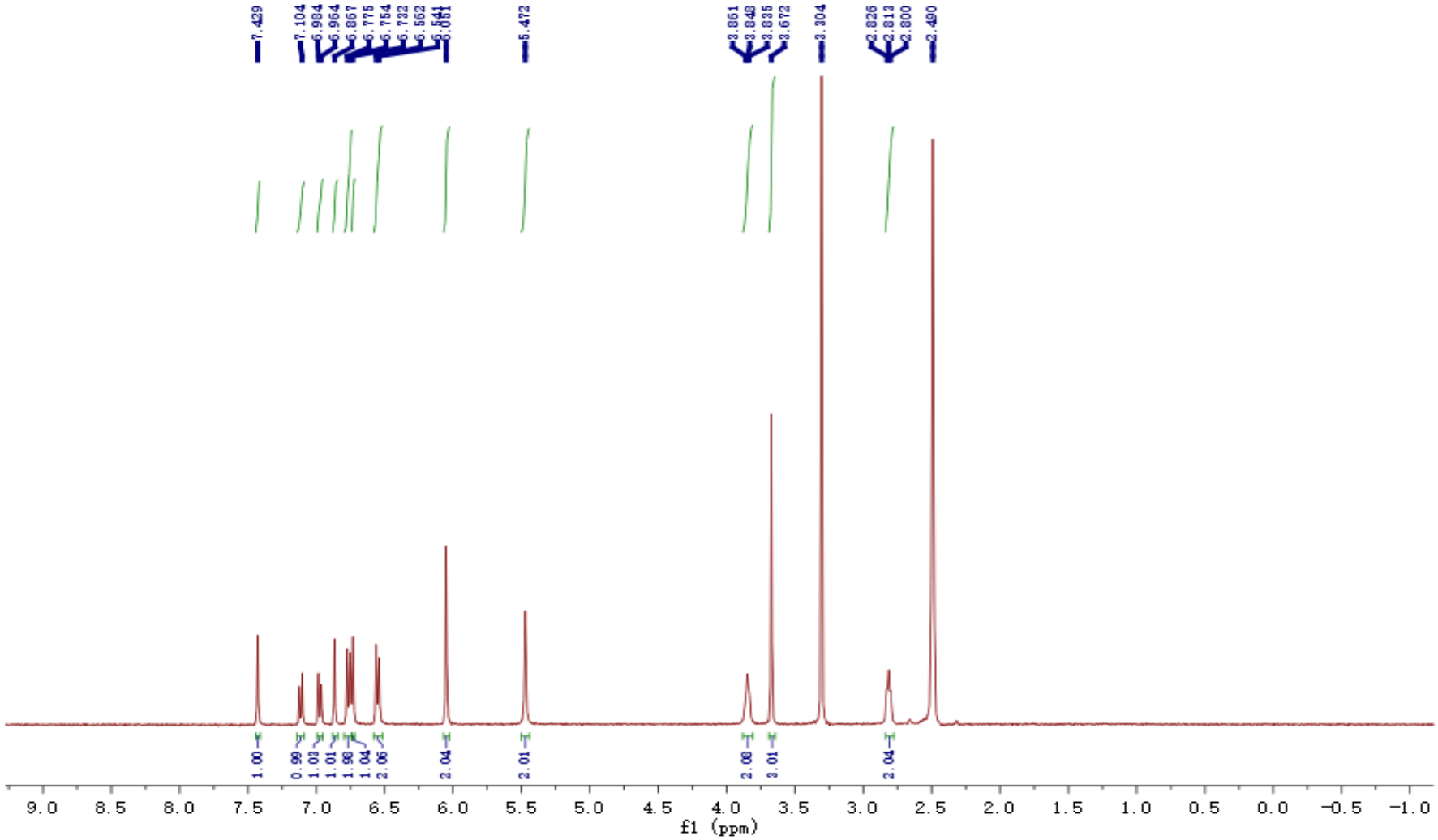

Figure S15. ^{13}C NMR (DMSO-d_6, 150 MHz) spectrum of **4f**.

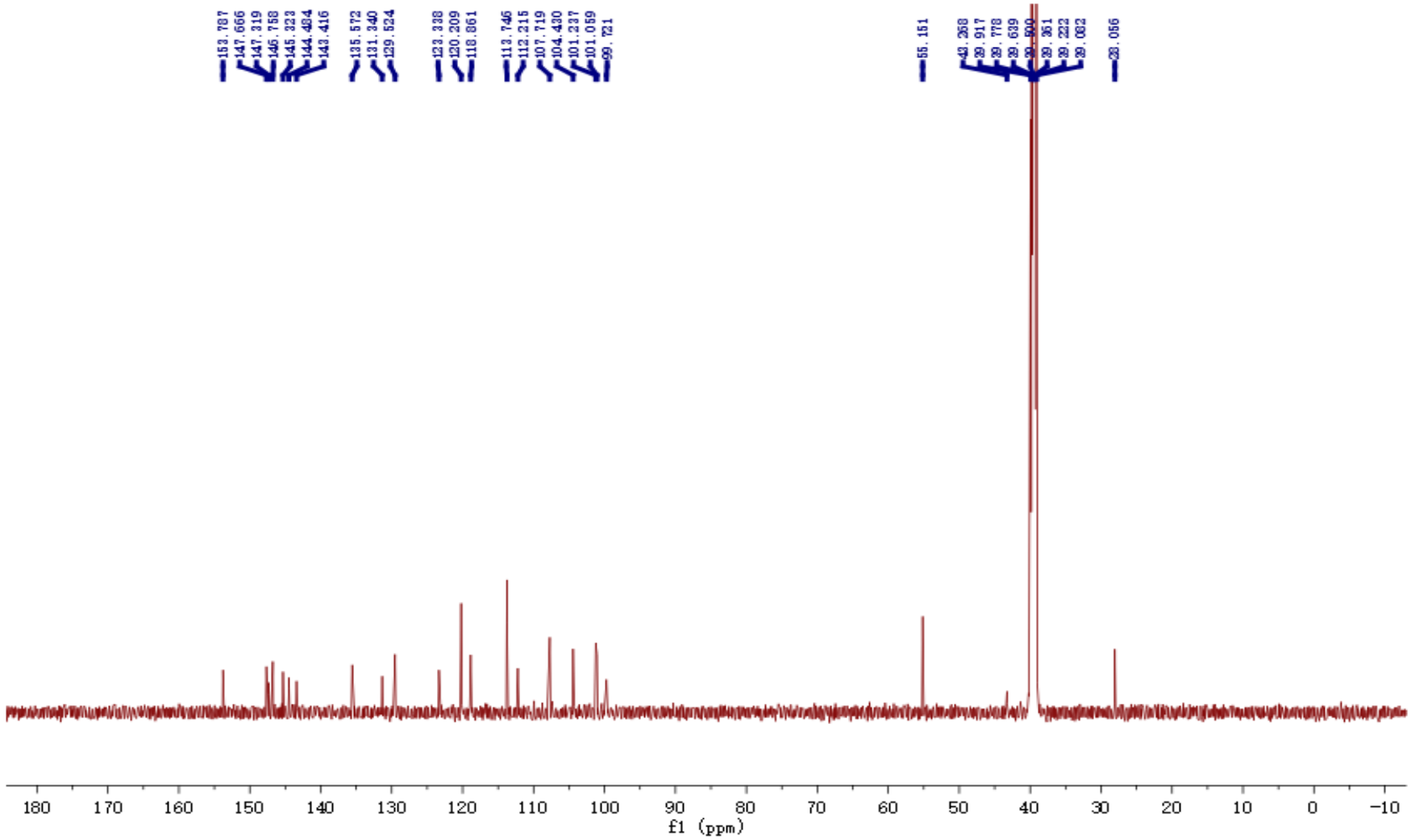

Figure S16. ^{1}H NMR (DMSO-d_6, 400 MHz) spectrum of **5**.

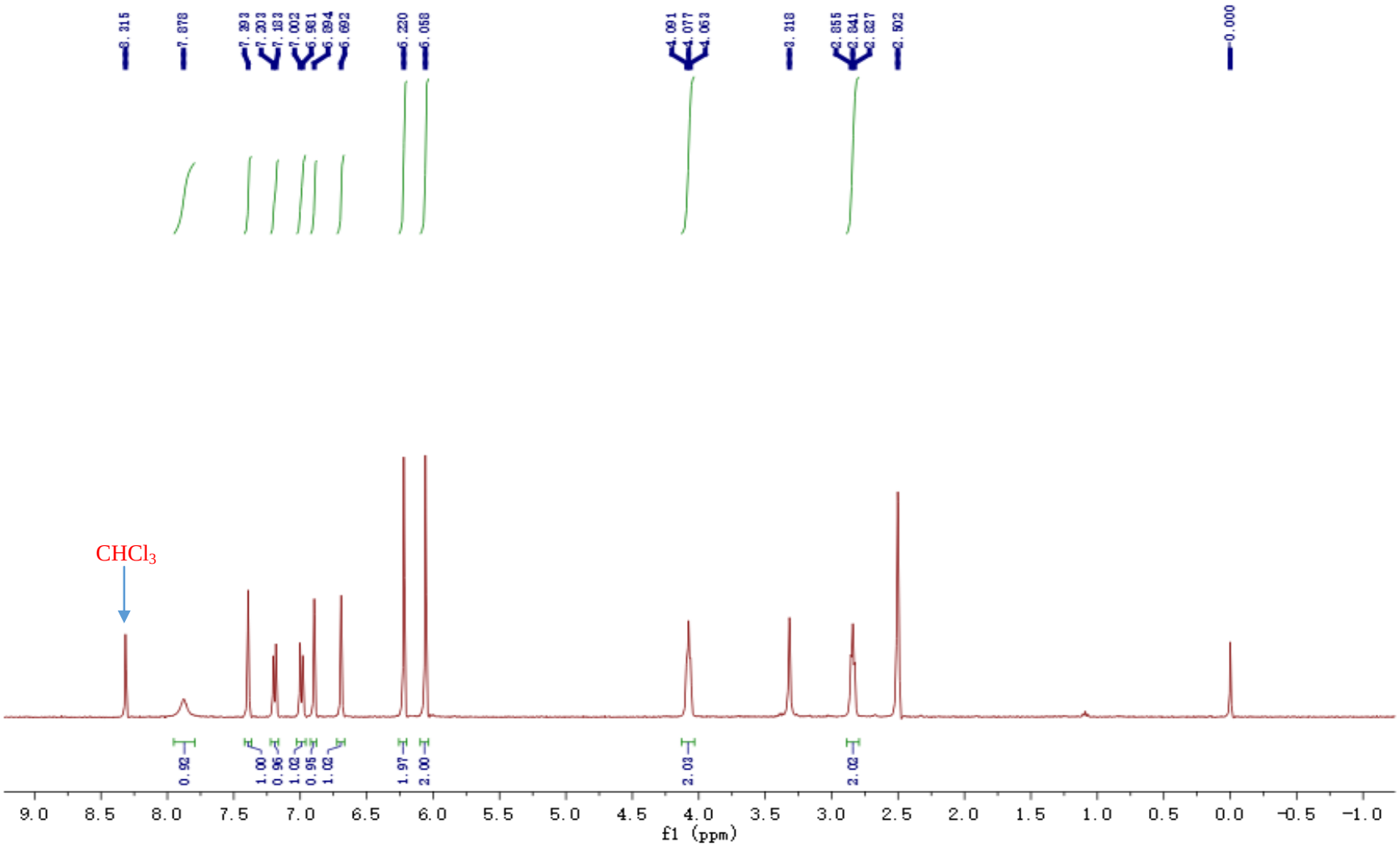

Figure S17. ^{13}C NMR (DMSO-d_6, 150 MHz) spectrum of **5**.

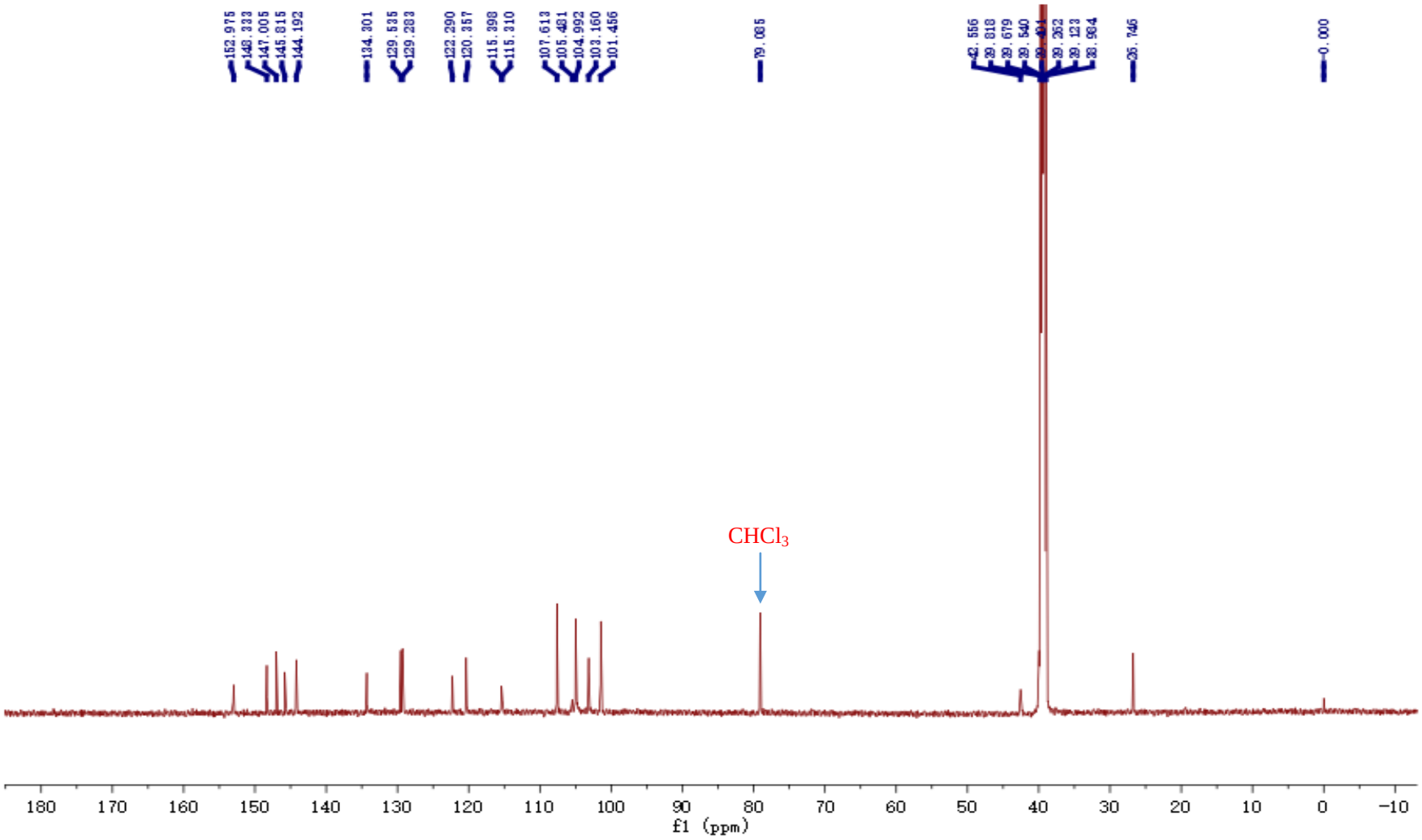

Figure S18. ^{1}H NMR (DMSO-d_6, 400 MHz) spectrum of **6a**.

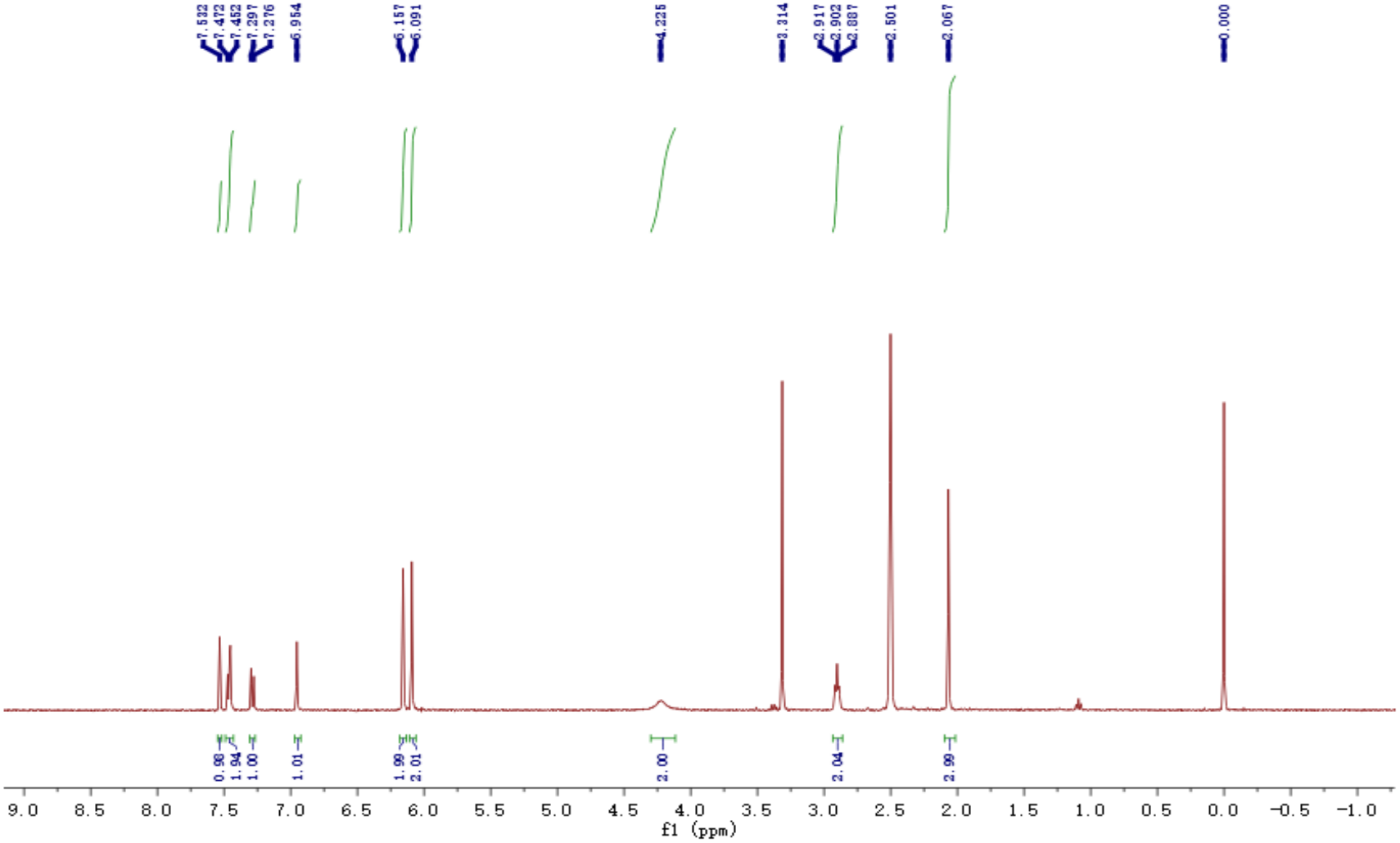

Figure S19. ^{13}C NMR (DMSO-d_6, 150 MHz) spectrum of **6a**.

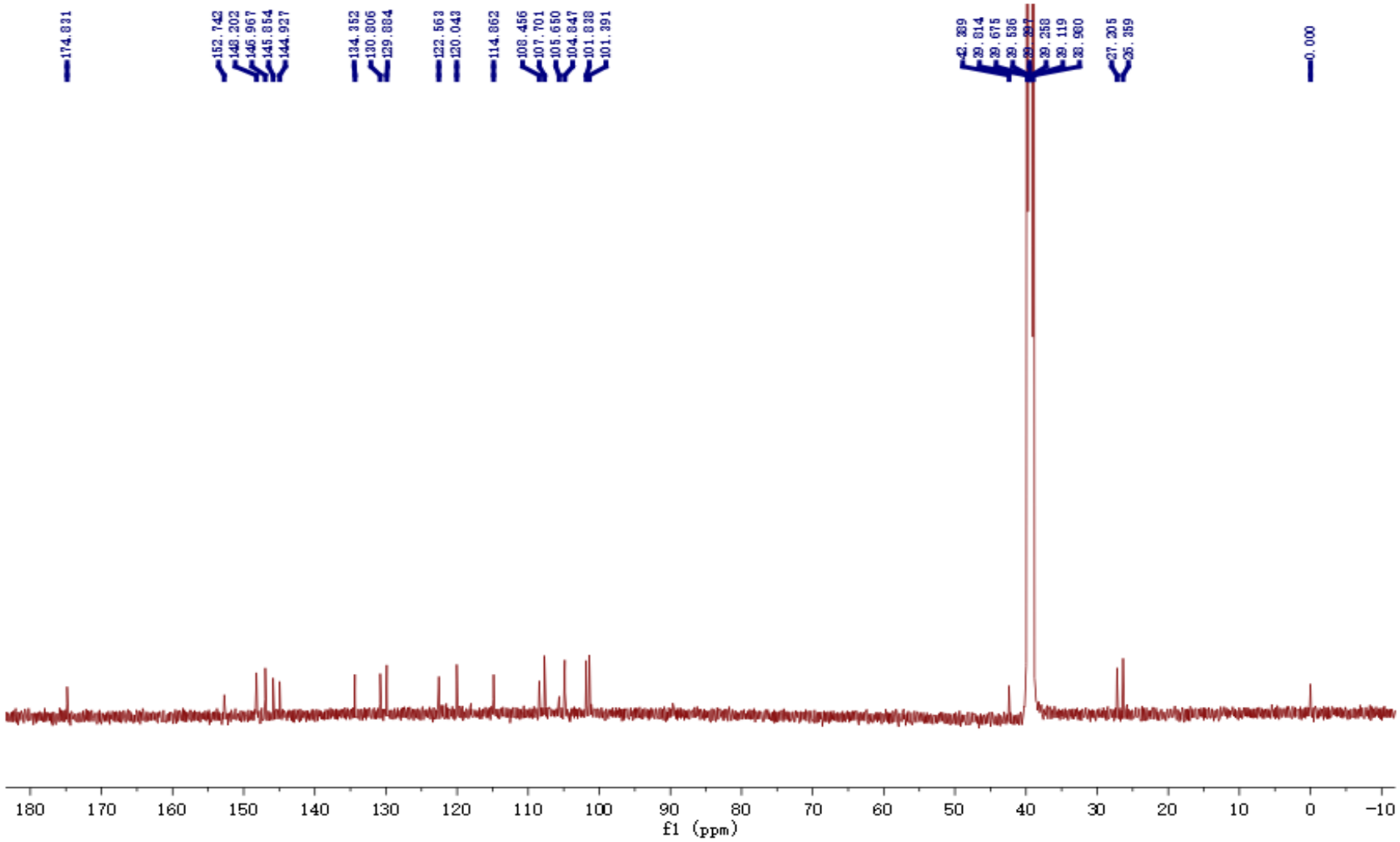

Figure S20. ^{1}H NMR (DMSO-d_6, 400 MHz) spectrum of **6b**.

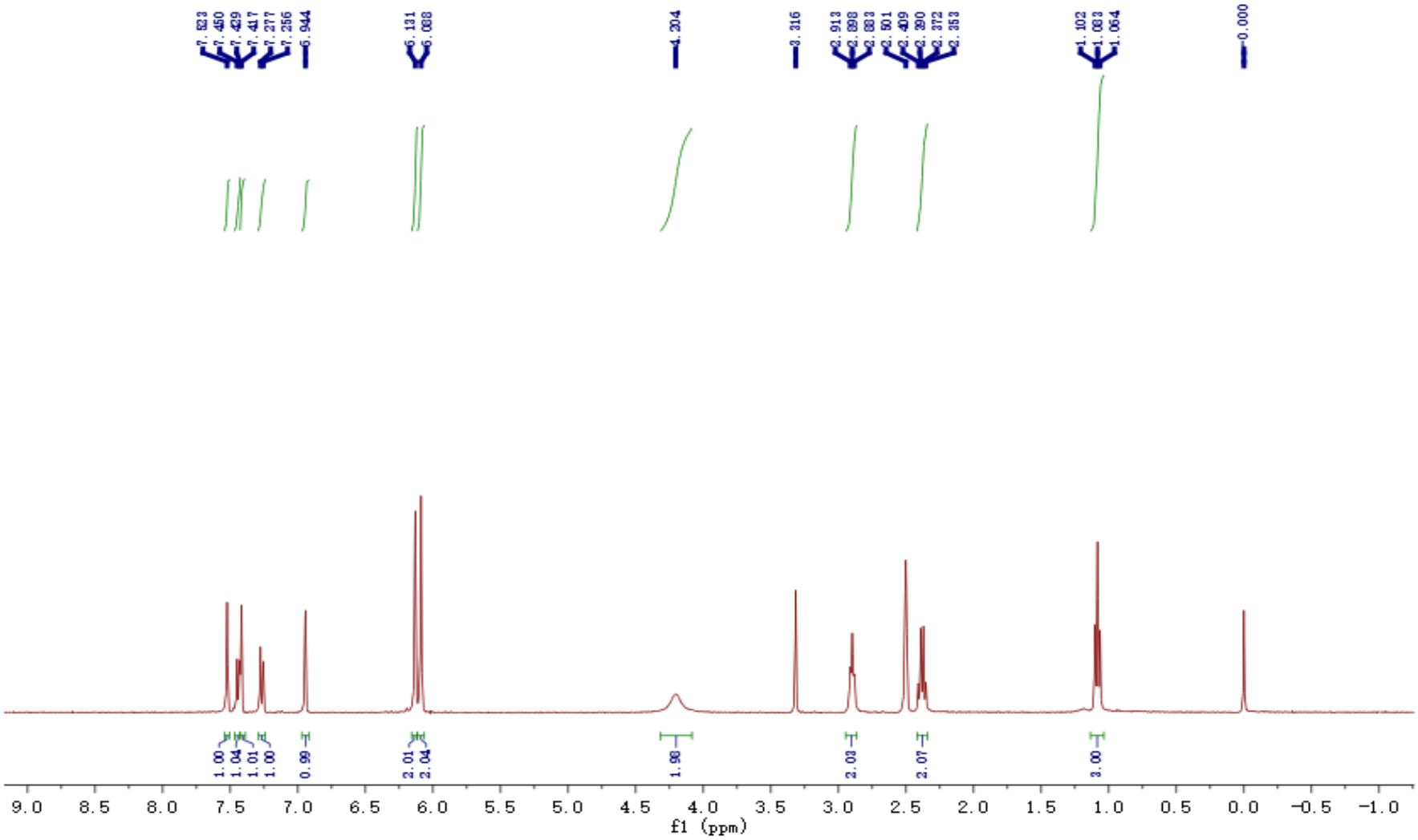

Figure S21. ^{13}C NMR (DMSO-d_6, 150 MHz) spectrum of **6b**.

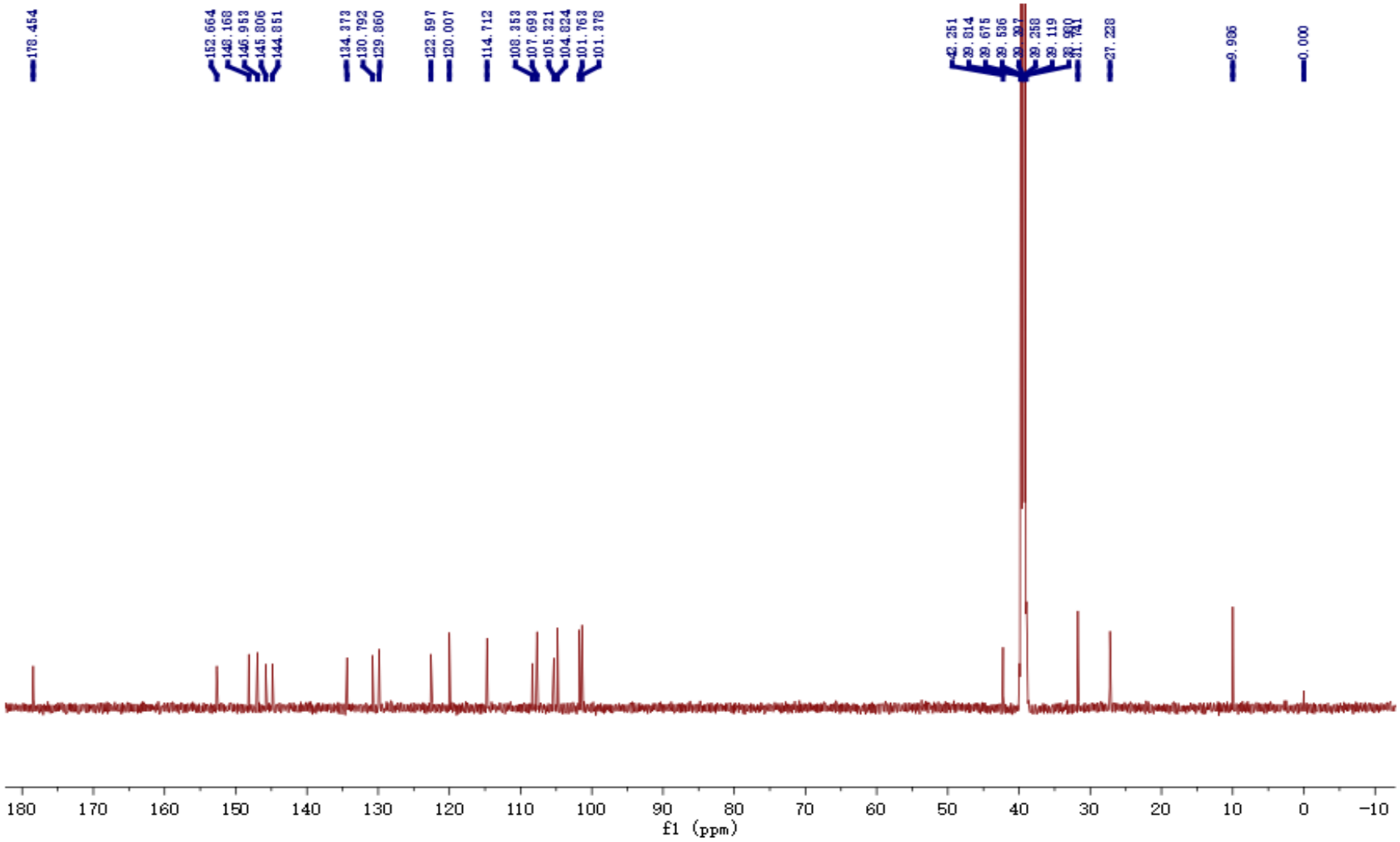

Figure S22. ^{1}H NMR (DMSO-d_6, 400 MHz) spectrum of **6c**.

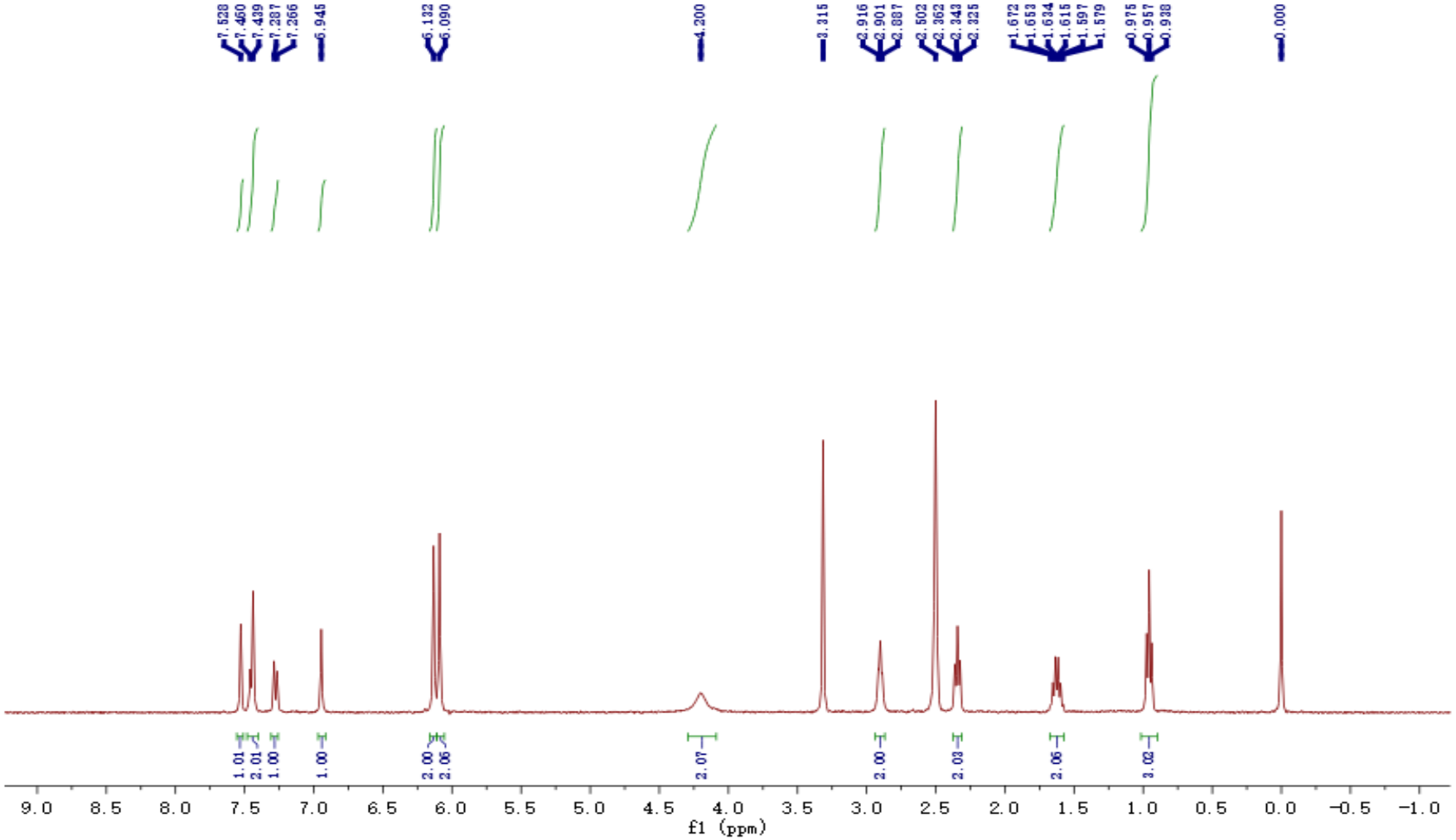

Figure S23. ^{13}C NMR (DMSO-d_6, 150 MHz) spectrum of **6c**.

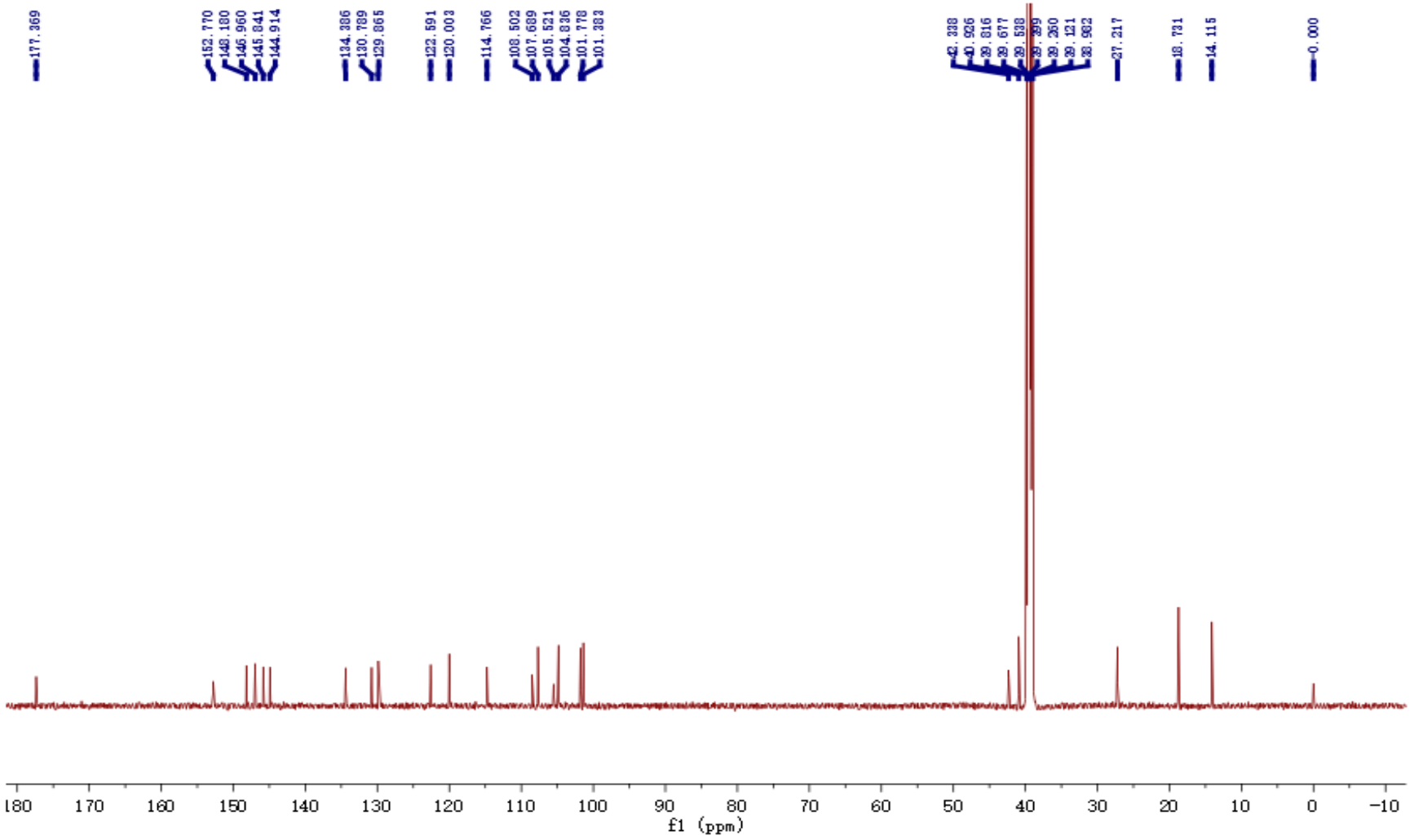

Figure S24. ^{1}H NMR (DMSO-d_6, 400 MHz) spectrum of **6d**.

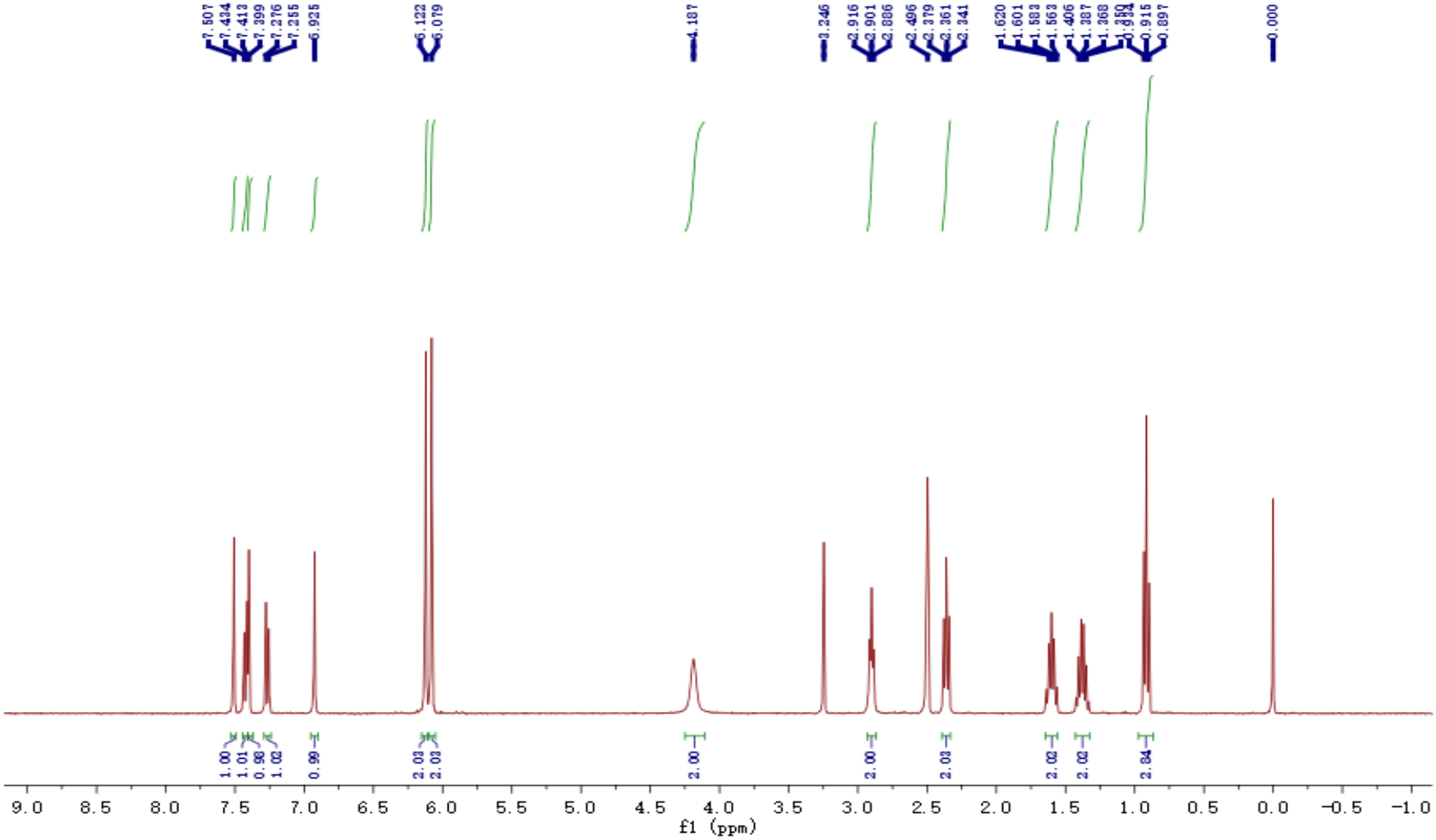

Figure S25. ^{13}C NMR (DMSO-d_6, 150 MHz) spectrum of **6d**.

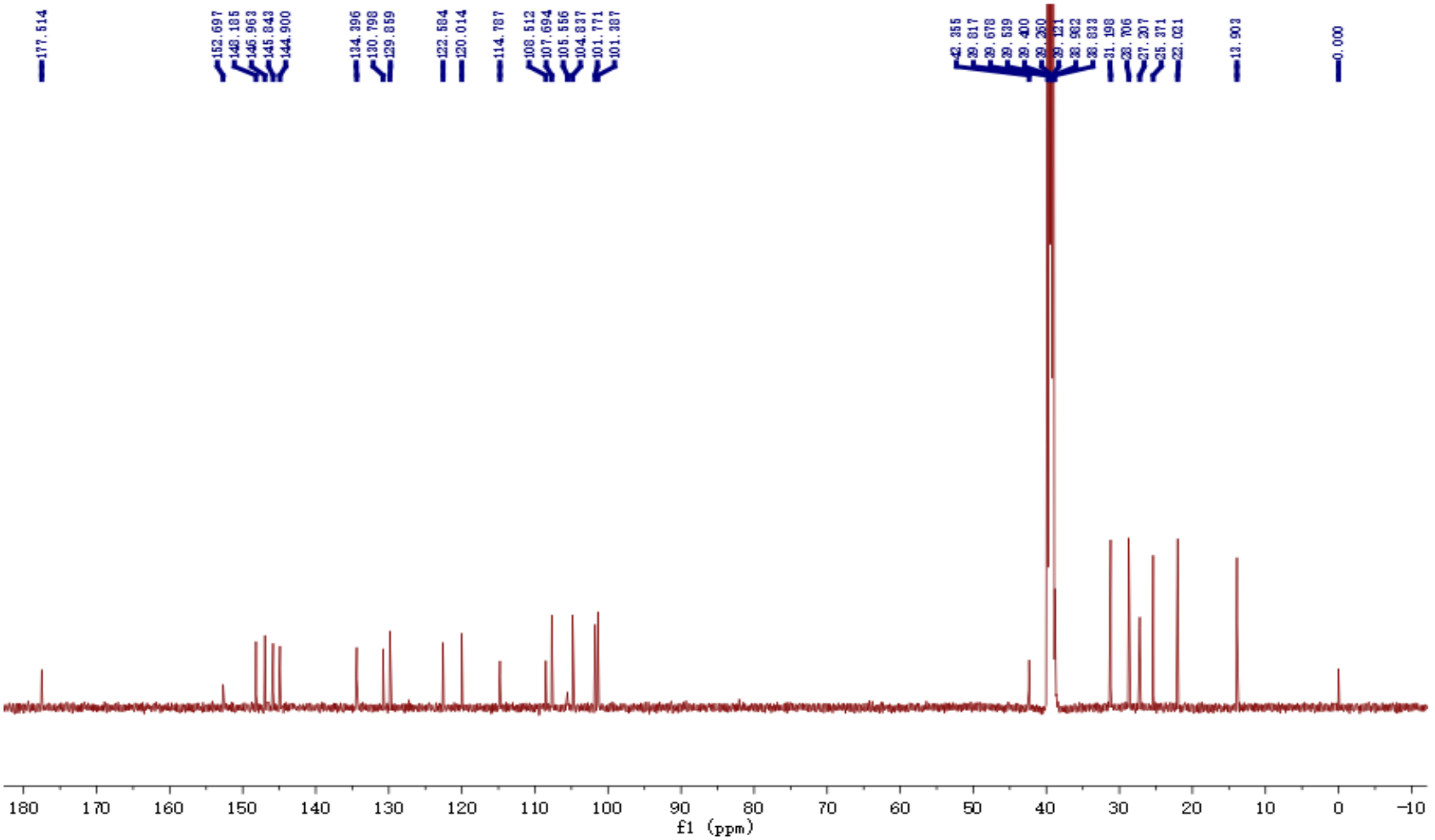

Figure S26. ^{1}H NMR (DMSO-d_6, 400 MHz) spectrum of **6e**.

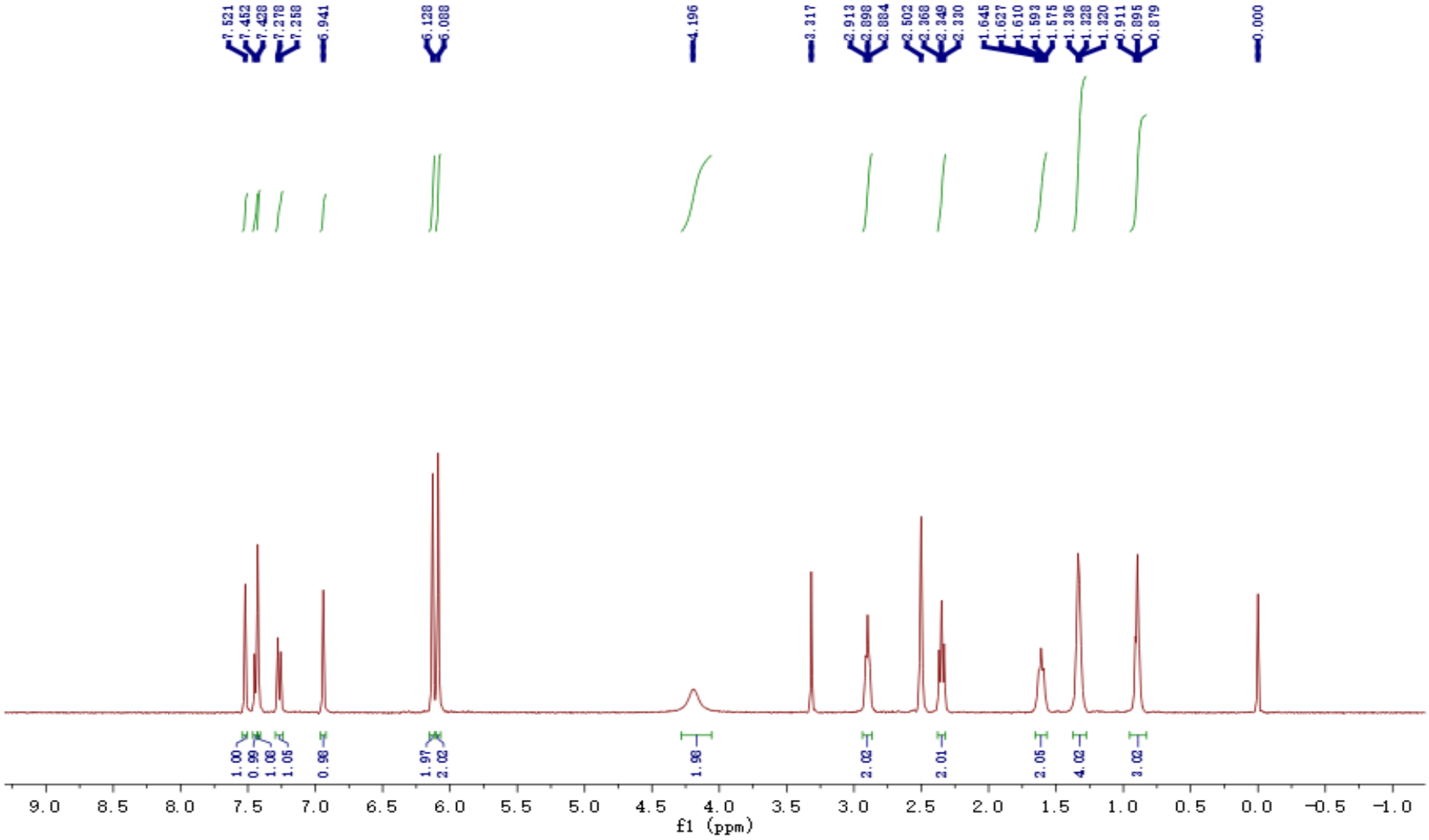

Figure S27. ^{13}C NMR (DMSO-d_6, 150 MHz) spectrum of **6e**.

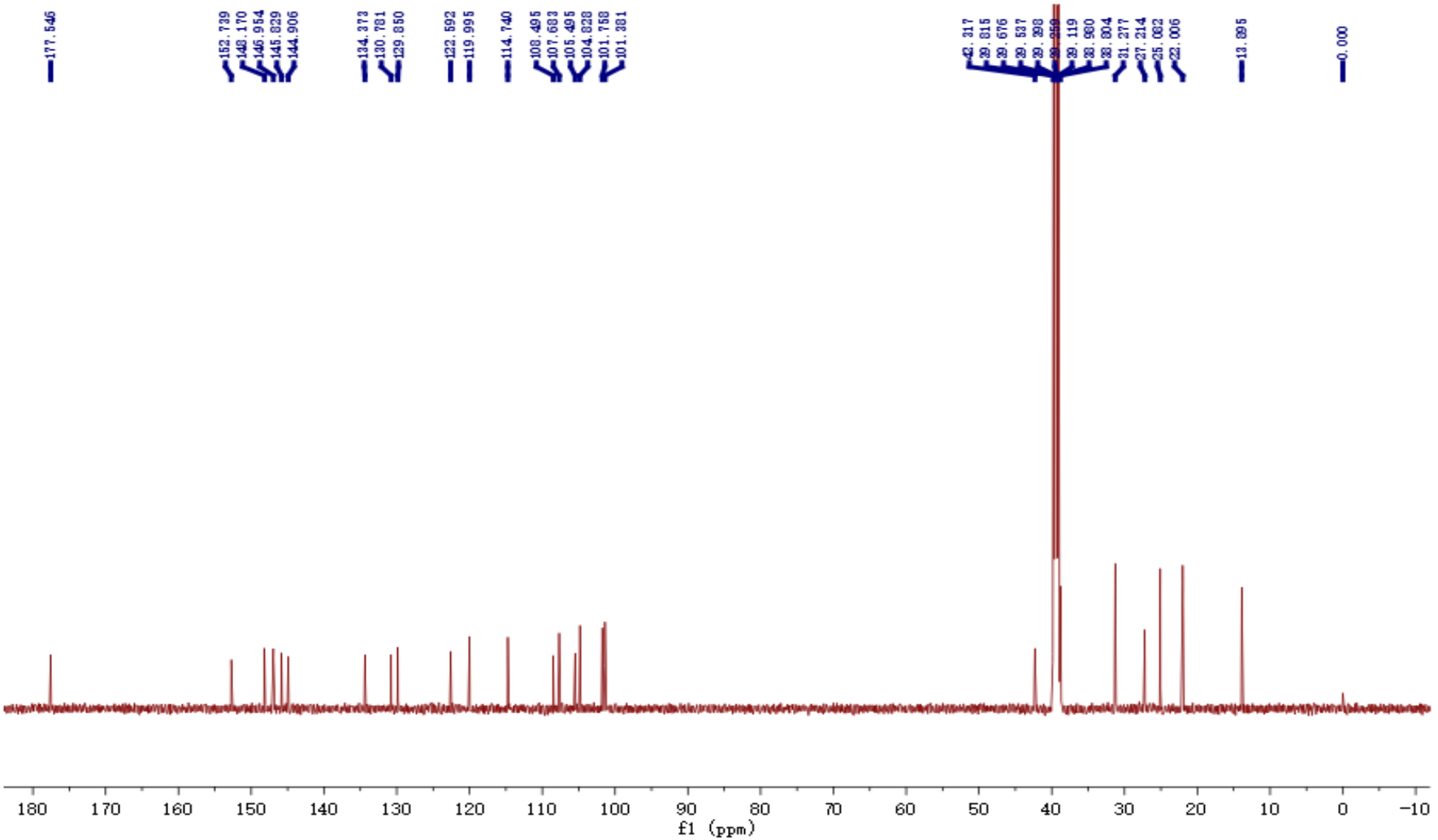

Figure S28. ^{1}H NMR (DMSO-d_6, 400 MHz) spectrum of **6f**.

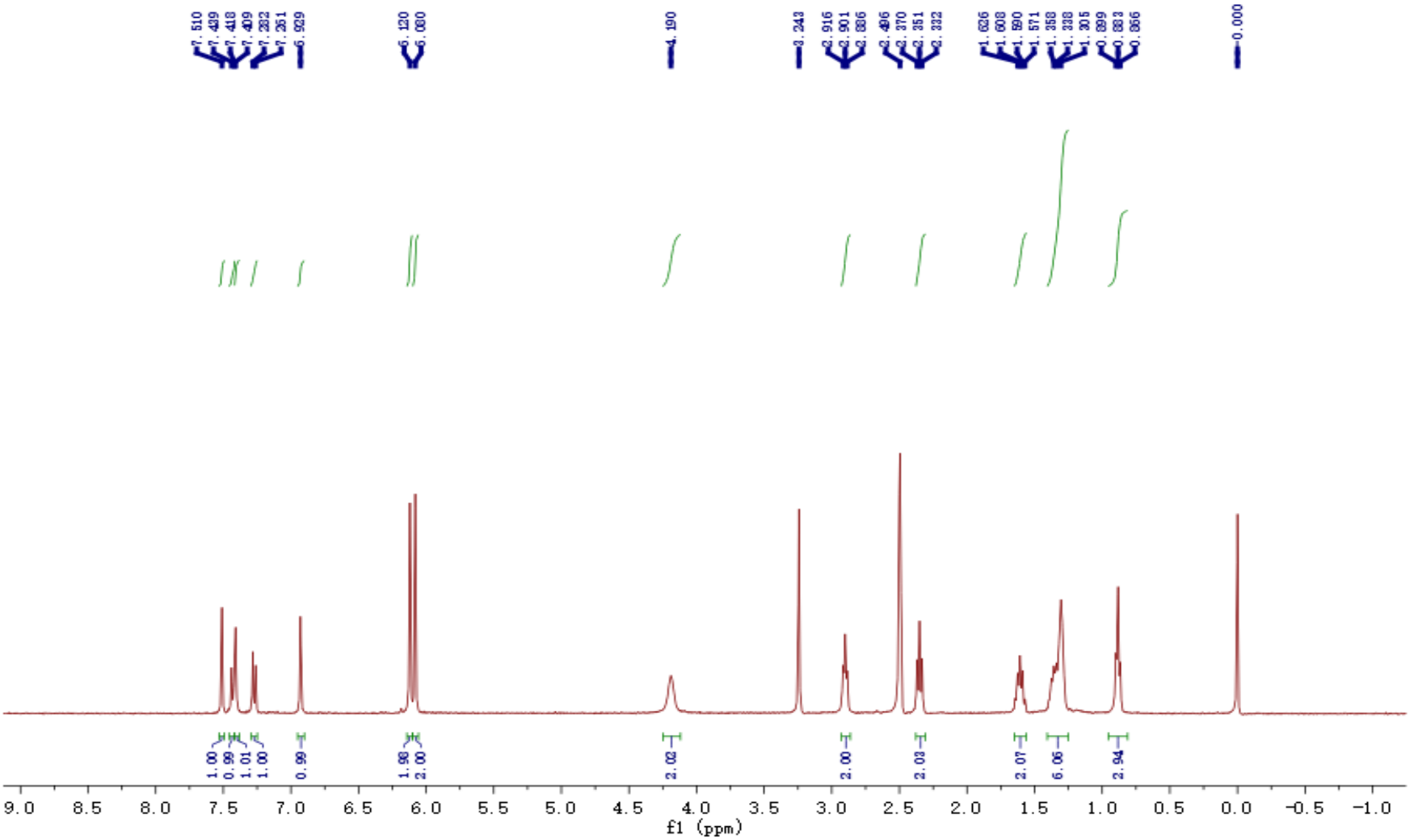

Figure S29. ^{13}C NMR (DMSO-d_6, 150 MHz) spectrum of **6f**.

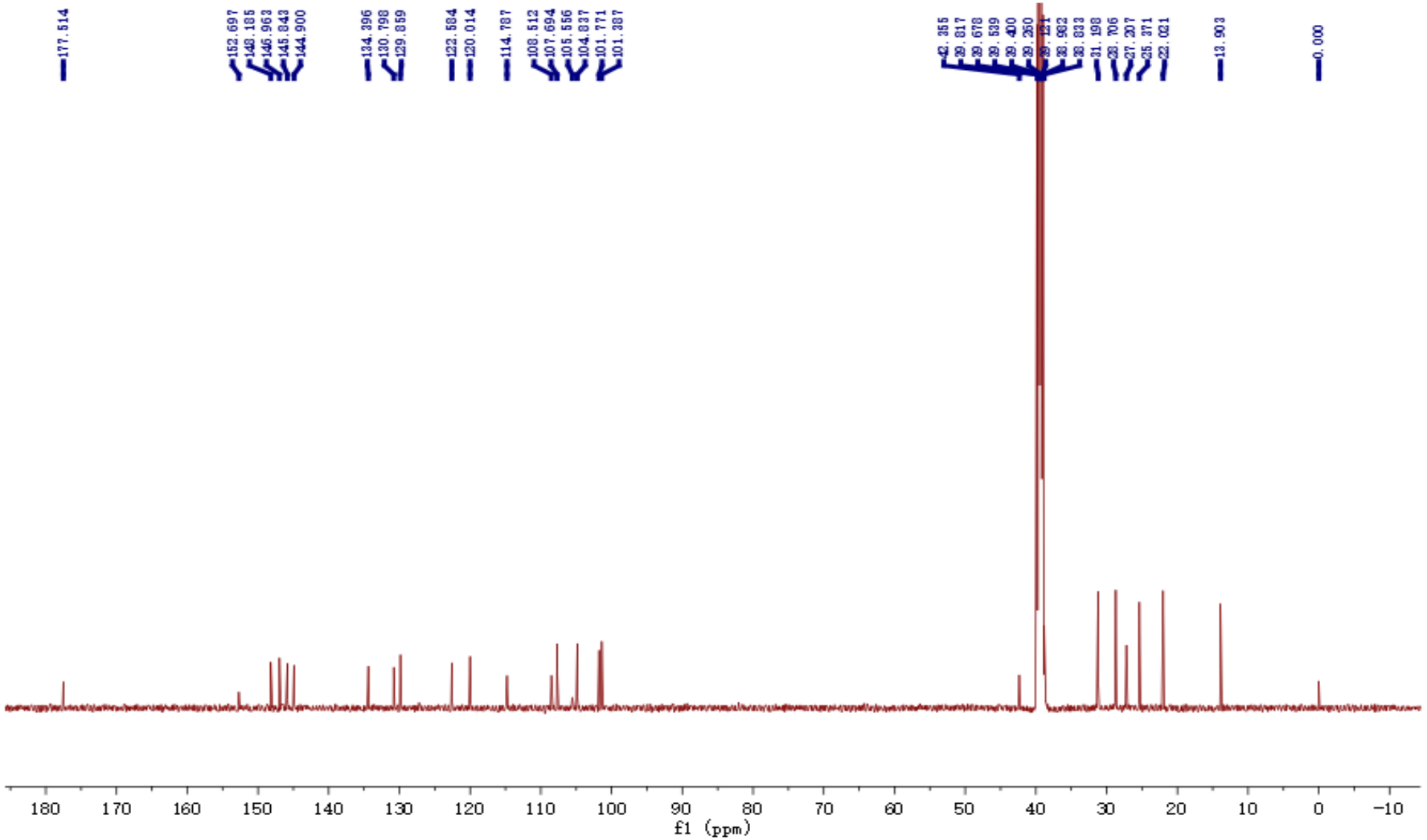

Figure S30. ^{1}H NMR (DMSO-d_6, 400 MHz) spectrum of **6g**.

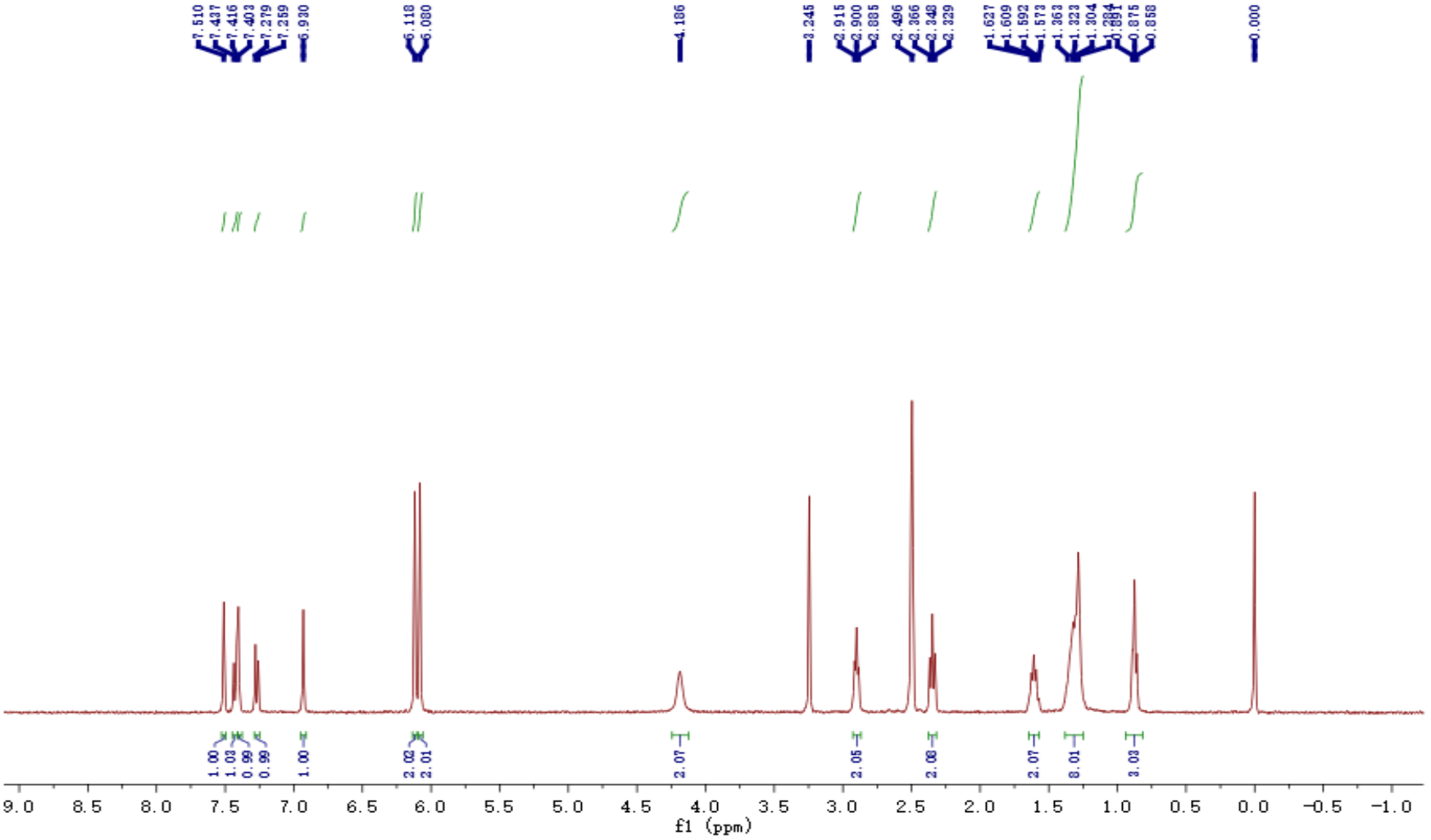

Figure S31. ^{13}C NMR (DMSO-d_6, 150 MHz) spectrum of **6g**.

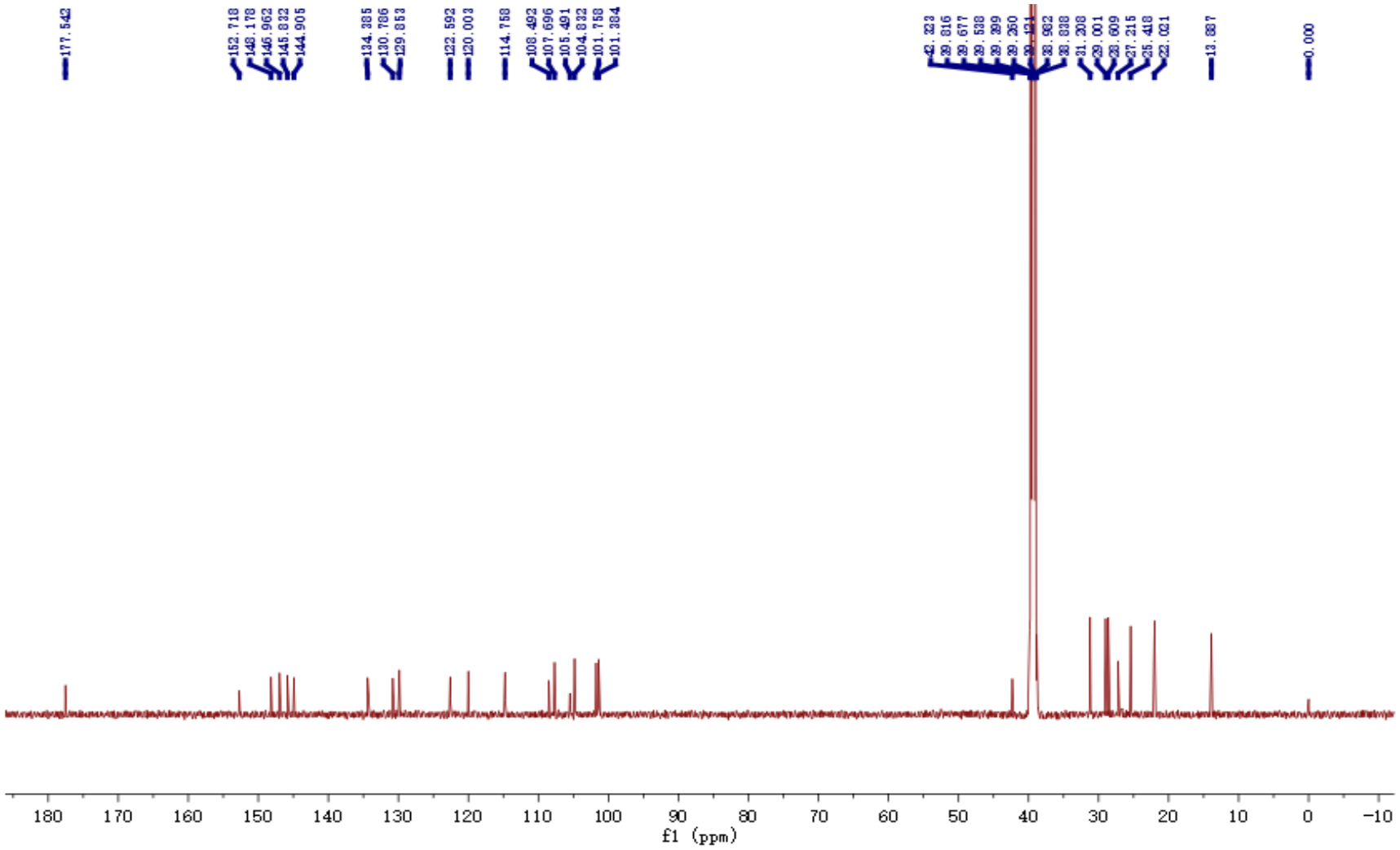

Figure S32. NOE (DMSO-d_6, 600 MHz) spectrum of **4d**.

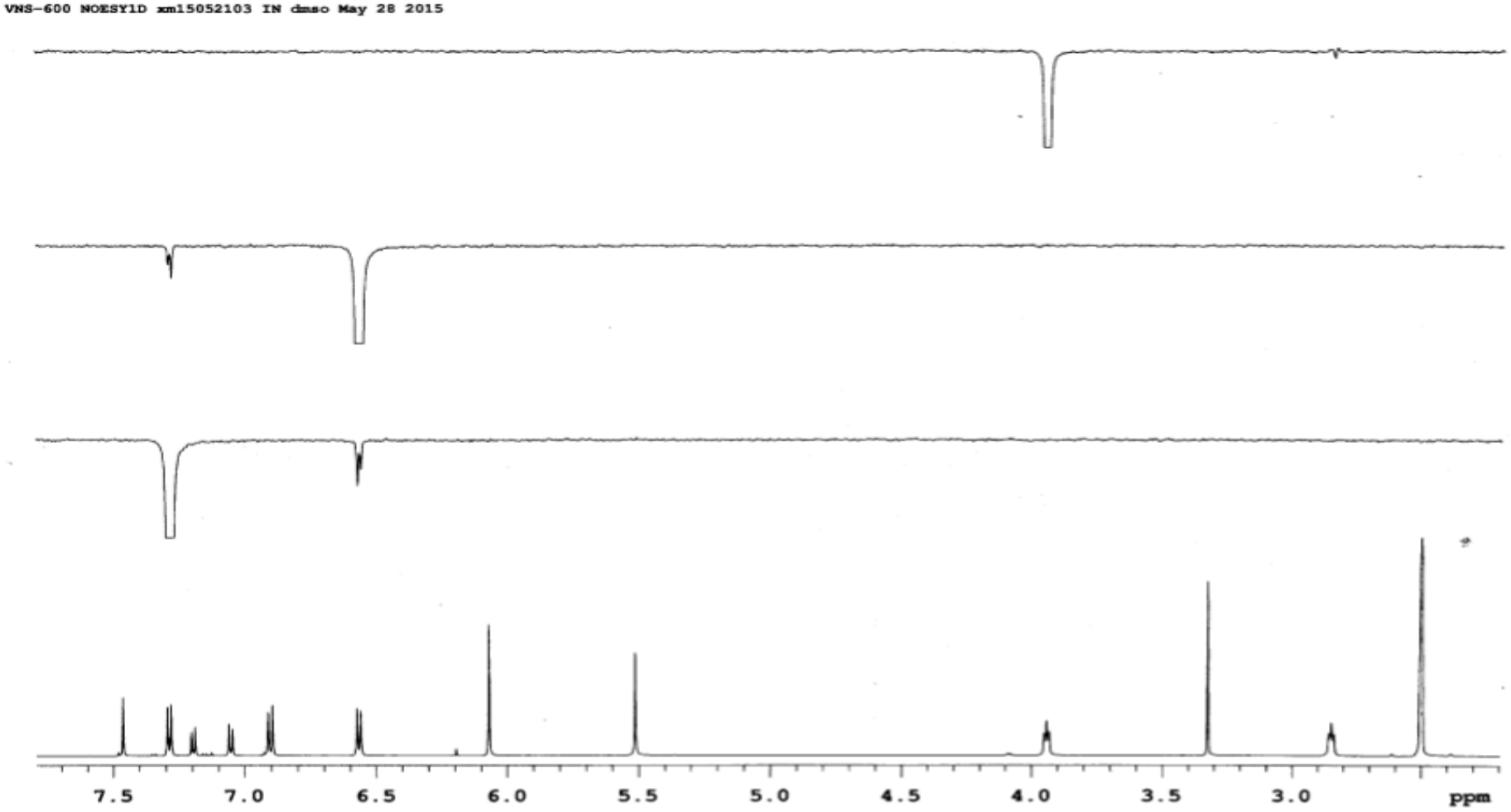

Figure S33. NOE (DMSO-d_6, 600 MHz) spectrum of **6c**.